INTERACTION OF TEMPERATURE, DISSOLVED OXYGEN AND FEED ENERGY ON ECOPHYSIOLOGICAL PERFORMANCE OF JUVENILE RED DRUM

A Dissertation

by

LANCE PIERRE FONTAINE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2008

Major Subject: Wildlife and Fisheries Sciences

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ABSTRACT

Interaction of Temperature, Dissolved Oxygen and Feed Energy on

Ecophysiological Performance of Juvenile Red Drum.

(May 2008)

Lance Pierre Fontaine, B.S., Tulane University;

M.S., Texas A&M University

Chair of Advisory Committee: Dr. William H. Neill

The red drum (*Sciaenops ocellatus*) is important for recreational fishing and aquacultural production in Texas' coastal waters and elsewhere in the nearshore Gulf of Mexico and in subtemperate to subtropical areas of the western North Atlantic Ocean. I performed indoor-tank and outdoor-pond experiments, in conjunction with automated respirometry and ecophysiological modeling, to assess interacting effects of temperature, dissolved-oxygen concentration (DO) and feed energy density on survival, growth, metabolism, and other measures of juvenile red drum performance.

The main objective was to test an energy/metabolism tradeoff hypothesis, which states that growth of fish exposed to high temperatures can be limited by available feed energy; whereas, growth of fish exposed to lower temperatures can be limited by their metabolic capacity to exploit available feed energy. Also, I examined the influence of DO on this relationship and evaluated the effects of cyclical regimes of temperature and DO on fish performance. Insights from laboratory-based feeding trials were incorporated in experiments conducted in hatchery ponds to assess effects of oxygen

supplementation and dietary additives—nucleotides and prebiotics—on performance in a more natural setting.

In examining these issues, various technologies were developed. These included a computer-based apparatus for autonomously inducing cyclical regimes of temperature and DO in experimental tanks over an extended period of time. Additionally, I developed a soft feed with low energy-density to simulate natural forage.

Experimental results supported the principal research hypothesis: At high temperature and DO, ecophysiological performance of juvenile red drum was enhanced by feeding to satiation with a high-energy feed (15.9 kJ/g) versus with a forage-simulating feed having lower energy density (4.1 kJ/g). Cyclical regimes of temperature and DO—as imposed in my particular laboratory experiments—did not impart growth benefits; however, the potential for enhanced growth via an appropriate cyclical environmental regime remains intact. Results from outdoor-pond experiments were consistent with laboratory results; however, the strong positive effect of feed energy density overwhelmed potential effects of dietary additives or oxygen supplementation on growth.

DEDICATION

I dedicate this to my parents Juanita and Peter,

my brother Gordon, my wife Eve, and Kaya.

ACKNOWLEDGEMENTS

This research was funded primarily by the Texas Sea Grant College Program. Additional financial support was provided by the Texas Water Resources Institute. Copyright permissions for Chapter III were conferred by the American Fisheries Society.

Numerous individuals and institutions were critical to the success of this research effort; to them, I give thanks and praise. First and foremost, I am very fortunate to have a strong committee composed of Drs. William H. Neill, Delbert M. Gatlin III, William E. Grant, F. Michael Speed, and Robert R. Vega. I could not have asked for a better committee; you are a respected group of exceptional scientists and philosophers who taught me to apply scientific principles and philosophy in a diverse and constantly varying world. I would like to acknowledge Dr. Neill, in particular, for taking a chance on admitting a young, budding scientist into his laboratory. Eight years later, I am richer by two degrees, innumerable positive and profound experiences, one heck of a good barbecue sauce, and a life-long friend.

Also, I am especially indebted to the Texas Parks and Wildlife Department (TPWD) as a whole for providing fish, facilities, expertise, and tremendous logistical support critical to the success of this study. This research would have been impossible without TPWD's assistance. In particular, I thank Robert Vega, Ruben Chavez, Paul Silva, and the rest of the outstanding staff at the CCA/CPL Marine Development Center (MDC) in Corpus Christi, TX; and, David Abrego and his staff at Sea Center Texas in Lake Jackson, TX.

I would like to express my gratitude to my technician John Wilson for his reliable and trustworthy assistance, his positive attitude, and his uncanny ability to attack problems from a different angle. Jon Goff, then at Texas A&M University's (TAMU) Aquacultural Research and Teaching Facility (ARTF), is acknowledged for his extensive technical, manufacturing, logistical, and culinary support throughout the duration of the experiments. Additional assistance was provided by Kasey Whiteman, Peng Li and Xena Wang, Gary Burr, Ken Webb, Scott Walker, Robbie Robinson, Kcal, and Kaya—your collective assistance and support is much appreciated. Scott Brandes provided the foundational programming and continued support for the respirometry equipment and environmental control apparatus. Kevin Clark was instrumental in realizing and constructing much of the respirometry equipment. Ken Davis, then at the U.S. Department of Agriculture's Harry K. Dupree National Aquaculture Research Center in Stuttgart, AR, assisted with analyses. Additional technical support for the cortisol assays was provided by Matt McEntire. Omega Protein, Inc. (Houston, TX) supplied the Special Select™ menhaden fish meal, Neptune™ fish solubles and menhaden oil. I also gratefully acknowledge International Ingredient Corporation (St. Louis, MO) for the donation of GroBiotic®A.

Over the years, many individuals from TAMU provided countless opportunities for philosophical, physical, mental, and spiritual development—critical components to the proper develop of a well-rounded graduate student. There are too many to list here; but I especially thank Neil Perry, Thomas Dixon, Pete Krawzcel, Dave LaFever, and Kevin Baker for opening my mind, body, and soul to the healing power of a bicycle.

My family—Mom, Dad, and Gordon—deserve acknowledgement for 30 years of emotional and financial support. At an early age they instilled in me a strong work ethic and a positive attitude. Truly, I would not have been able to do this without you.

And last, but perhaps most importantly, I thank my wonderful wife Eve. Her ceaseless and unquestioning motivation and support made the extra effort for a doctoral degree possible. It was a long journey but we both made it!

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CHAPTER I

INTRODUCTION

The red drum (*Sciaenops ocellatus*) is native to the Gulf of Mexico and to temperate and subtropical parts of the western Atlantic Ocean. This sciaenid, commonly referred to as redfish or channel bass, is an important euryhaline sport and food fish, especially in the Gulf-coast states of the U.S. and Mexico. Commercial and recreational exploitation of the red drum stock in the Gulf of Mexico intensified markedly in the years between World War II and the mid-1970s (Boothby and Avault 1971; Matlock 1990; McEachron et al. 1993). During the early-mid 1980's, the U.S. and several Gulf-coast states, including Texas, prohibited the commercial harvest of red drum from the Gulf of Mexico, to counter what by then had become a drastic stock decline (Matlock 1990). Now, the red drum is cultured both for stock enhancement (McEachron et al. 1993) and for food-fish production (Wurts and Stickney 1993).

Red drum and other sciaenid fisheries continue to serve as an integral part of Gulf Coast tourism, economy, and culture. Recent estimates indicate that saltwater angling generates $1.3 billion annually and provides over 13,000 jobs for the state of Texas (Sturdevant 2005). Despite current harvest regulations and the positive sociological and economical aspects of the fishery for this sciaenid, there remains concern about its sustainability. A recent National Marine Fisheries Service (2004)

This dissertation follows the style of Transactions of the American Fisheries Society.

report indicated that Gulf of Mexico populations of red drum are currently "overfished" (i.e. stock size is below a prescribed biomass threshold) and suffering from "overfishing" (i.e. rate of harvest is above a prescribed fishing mortality threshold). Further, a recent estimate of average yield from the Gulf of Mexico (based on annual yield from 1994 – 1996) was determined to be over 5000 metric tons annually, roughly twice the current sustainable yield for this species (NMFS 1999).

In an effort to maintain the fishable stock of red drum in the western Gulf of Mexico, Texas Parks and Wildlife Department (TPWD) engages in a very large-scale stock enhancement program (Procarione and King 1993; Vega et al. 2003; Vega et al. 2007). Since the mid-1980s, almost half a billion juveniles of this important sport and food fish have been produced in TPWD-operated hatcheries and released into Texas bays (Vega et al. 2007). TPWD scientists strive to utilize available information from research, as well as conduct studies of their own, as an integral part of their effort. Their strategy of selecting broodstock and stocking localities based on genetic evidence of regional sub-populations of red drum exemplifies these efforts (Gold 1999; Turner et al. 2002). In keeping with their philosophy, it is crucial to continue researching improvements upon existing methodologies in order to further enhance the production and beneficial impact of these stocked fish in Texas coastal ecosystems. Thus, by assessing abiotic effects and their interactions on red drum physiology, I hope to bolster the effort to restore and maintain Gulf of Mexico populations of red drum at sustainable levels.

INCORPORATING ECOPHYS.FISH

As coauthor, I have helped develop and test an ecophysiological model that can account for about 80% of the variation in growth rates of juvenile red drum caged in pond and bay habitats, as a function of initial fish size, feed quantity and quality, temperature (Ta), dissolved oxygen concentration (DO), salinity, pH, and residual environment (Neill et al. 2004). The effect of residual environment is estimated as variation in marginal metabolic scope (MMS; Neill and Bryan 1991), via automated routine respirometry (Springer and Neill 1988; Forsberg and Neill 1997; Fontaine 2002; Fontaine et al. 2007). MMS, a concept critical to this model, is the ratio of routine metabolic rate to the DO limiting for that rate, and has proven a reliable correlate of metabolic scope for growth (MSg) of fish. The relative impacts of variation in residual environment and the other, more conventionally measured components of environment are assessed by integrating environmental data and results of the MMS assay into a simulation model called "Ecophys.Fish."

Ecophys.Fish, which represents another key component of this approach, is a computer-based ecophysiological model of red drum performance. Using Ecophys.Fish one can simulate the impact that interactions of temperature, dissolved-oxygen concentration (DO) and food energy-density have on the survival, growth, and metabolic performance of juvenile red drum. Thus, Ecophys.Fish allows for the generation of testable hypotheses and prediction of the results of potential management regimes designed to increase size and hardiness of hatchery-produced juvenile red drum being released by Texas Parks and Wildlife for stock enhancement. Furthermore,

Ecophys.Fish and the ecophysiological approach provide the platform with which data and results may be interpreted and further explored for complex relationships.

Interactions of environment and nutrition

Physiologically, various factors interact to influence the growth and overall health of fish; these include but are not limited to environmental conditions ("water quality"), a proper balance both of nutritive and non-nutritive dietary components that enhance immunity and disease resistance, and feeding practices (Gatlin 2002a). In particular, fish performance reflects a dynamic balance between supplies of oxygen and energy-yielding substrates for metabolism (Fry 1947; Brett 1979). The balance has been set by evolution to give best performance under an individual's optimum environmental and physiological circumstances. Increased feed energy tends to shift performance optima towards higher temperatures, where more metabolic scope is available (Fry 1947; McLaren 1963; Brett 1971; Brett 1979; Azevedo et al. 1998; Gillooly et al. 2001; Neill et al. 2004; Fontaine et al. 2007). Some studies have suggested that optimally cycling Ta regimes may further increase metabolic scope in certain fish species (Hubbs 1964; Hokanson et al. 1977; Dickerson and Vinyard 1999). Simulations with Ecophys.Fish indicate that for red drum this extra metabolic capacity is useful only if available feed energy also is increased, perhaps to levels above approximately 10.5 kJ/g.

Few natural forages available to red drum have this much energy, but many prepared feeds have over 16.7 kJ/g and some even more than 20.9 kJ/g. To further enhance the organism's ability to better use these high-energy feeds, they must be made more functional than those commercially available at present. Increased functionality is

accomplished by supplementing diets with nucleotides, beta-glucans, and probiotics—such as yeast and bacteria—that have the potential to enhance metabolic "health"—and consequently, metabolic scope for growth—beyond normal levels. Previous studies support this concept; under ideal environmental and nutritional conditions, red drum are capable of achieving sizes from 1 to 2 kg in one year (Luebke and Strawn 1973; Gatlin 2002b; Vega 2003). Comparatively, red drum in the wild eating natural foods, typically reach only about 0.45 (Simmons and Breuer 1962) to 0.8 kg in one year (Goodyear 1989).

While still a relatively new strategy, the inclusion of nucleotides and probiotics in aquacultural feeds has proven beneficial to various species of fish. Gatlin (2002a) provides an extensive review of the general health benefits afforded to fish via the addition of non-nutritive materials to the diet. Research conducted on various salmonid species has demonstrated improved disease resistance, reduced mortality following challenges, and enhanced growth rates when feeds are amended with beta-glucans and nucleotides (Burrells et al. 2001a; Burrells et al. 2001b). Li and Gatlin (2003) observed lower mortality and enhanced weight gain, higher feed efficiency, and greater resistance to *Streptococcus iniae* in hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed a diet supplemented with brewer's yeast (*Saccharomyces cerevisiae*). Also, studies with Nile tilapia (*Oreochromis niloticus*) have suggested that yeast additives can stimulate growth in this species (Lara-Flores et al. 2002). The effects of these additives on the survival, growth, and performance of red drum have yet to be thoroughly studied.

GENERAL HYPOTHESES AND PREDICTIONS

The central hypothesis examined here is reminiscent of McLaren's (1963) "energy bonus hypothesis," which suggests that diel-cyclic exposure to low temperature can impart an energetic savings to vertically migrating aquatic organisms. Brett (1971) acknowledged that this form of behavioral energy conservation is present in organisms such as bats and birds. Despite this, he concluded that the energy bonus hypothesis—as it might apply to salmonids—is likely to be valid only when energy intake is limiting. Behavioral thermoregulation to maximize the growth benefits of an insufficient energy supply may be the most prominent mechanism by which to induce energy-saving migrations. Nevertheless, the argument that mobile poikilotherms migrate to lower temperatures to maximize bioenergetic efficiency remains tenuous. It is possible that previous attempts to test the "energy bonus hypothesis" for fishes have been compromised and unfairly discredited by a lack of treatment optimization and, perhaps, also by peculiarities of stenotherm bioenergetics (Brett 1971). Such optimization requires explicit predictions, which Ecophys.Fish can produce via simulation of the underlying physiological processes.

Ultimately, both McLaren's energy bonus hypothesis and the energy maximization hypothesis evaluated here devolve upon metabolic scope for growth—MSg being defined as the difference between active metabolic rate and routine metabolic rate. The hypothesis developed by McLaren and Brett, however, is functionally distinct from the energy/metabolism-tradeoff hypothesis invoked by Ecophys.Fish (Neill et al. 2004); the former suggests a physiological attempt to conserve energy and maintain

MSg in energy-limited situations. Exposure to lower temperature reduces various temperature-dependent activities, particularly spontaneous locomotion, thus reducing routine metabolism (McLaren 1963; Brett 1971). Effectively, this preserves enough MSg to allow the fish to continue directing the yields from limited food resources into growth.

In contrast to the above, energy/metabolism-tradeoff acts to increase MSg in non-energy-limited situations by increasing metabolic capacity to process food. When fish are exposed to a cyclical temperature and dissolved oxygen (DO) regime their DO—and temperature, although less so—acclimation state decreases, thereby increasing active metabolic rate (Neill et al. 2004). Consequently, the fish can utilize more of its metabolic scope to fully exploit the energetic and nutritional benefits of a nutritionally adegquate feed that has high energy density. In simulation models, the end result is an increase in growth rate over 1) fish that are fed low-energy feeds, as well as 2) fish that are exposed to other regimes of temperature and DO, either cyclical or temporally constant.

OBJECTIVES

The dissertation chapters are written as individual manuscripts for scientific publication. Thus, specific research objectives are presented in each chapter, with some redundancy of background information among chapters.

My overall research goal was to achieve new scientific insight into how interactions of environment and nutrition affect fish growth and metabolism. Results and outcomes of my research could have a major positive impact on efficiency of

juvenile red drum production either for stock enhancement or for food-fish aquaculture—a better understanding of the interaction of abiotic and nutritive factors on fish growth would aid hatchery managers in providing conditions for optimal production of red drum.

A recurrent feature of my experiments was the contrast between a newly developed soft feed with low energy-density—to simulate natural forage—and a standard high-energy feed. Since "real" natural forage is subject to regional and seasonal variation in availability and composition, these feeds were developed to provide nutritional consistency at each energetic level across each experiment. The low energy diet (LE) contained ~ 4.1 kJ/g (or 980 cal/g), ~ 80 % moisture, and was empirically determined to have an energy digestibility of ~ 74.8 % in red drum. The high energy diet (HE) was a dry pelleted feed with ~ 15.9 kJ/g (or 3800 cal/g), ~ 10 % moisture, and an empirically determined energy digestibility of ~ 72.9 % for red drum. The feeds are described in more detail in Chapter III.

I) The first objective was to test the hypothesis that growth of fish exposed to high temperatures can be limited by available food energy; whereas, growth of fish exposed to lower temperatures can be limited by their metabolic capacity to exploit available food energy. This "energy/metabolism-tradeoff hypothesis" was evaluated in the laboratory by exposing juvenile red drum to two levels of dietary energy, LE and HE, and to three temperatures-- ~19, ~25, and ~29°C-- for a period of 6 weeks. Growth rate and metabolism were evaluated at termination of the study and Ecophys.Fish was employed to elucidate

experimental results potentially confounded by interactions between fish weight and the controlling effects of temperature on metabolism. A follow-up, 6-wk-long experiment was performed to confirm results for fish fed the two diets at ambient temperature (~26°C) and to further resolve responses by examining body-condition indices and proximate composition. Additionally, fish were assayed for differential cortisol response to net-confinement stress.

II) The second objective was to determine the influence of DO on the energy/metabolism-tradeoff hypothesis and to evaluate the effects of cyclical regimes of temperature and DO on fish performance. To assess the former, juvenile red drum consuming two levels of dietary energy (LE and HE) were exposed to three "static" environmental regimes in the laboratory: 1) two levels of temperature (18.5 C and 28.5 C) with DO at air-saturation; 2) two levels of DO (low DO = 25-40 % air-saturation, and high DO = near 100 % air-saturation) at a constant water temperature of ~ 18.5 C; and 3) two levels of DO (low DO and high DO) at a constant water temperature of ~ 29 C. The latter objective was evaluated in the laboratory by cycling temperature from ~ 20 to ~ 28 C and dissolved oxygen between low DO and high DO over a 24-h period. After 4 weeks, growth rate and metabolic performance were measured. Experimental results were analyzed statistically and evaluated using Ecophys.Fish.

III) The third objective expanded the scope of research to evaluate juvenile red drum performance in a hatchery-pond setting. In particular, potential effects of oxygen supplementation and dietary additives on survival, growth, and metabolic performance were examined. Two different cage studies were conducted in culture ponds at a Texas Parks and Wildlife Department hatchery facility. In the first study, the commercial prebiotic GroBiotic®A was added as a supplement to the two experimental diets (LEg and HEg). Performance of fish receiving the supplemented diets was compared to that of fish consuming the non-supplemented, or basal, diets (LEb and HEb). In the second study, in order to maintain DO above limiting levels, gaseous oxygen was administered during evening hours to caged red drum receiving the basal formulations of the two experimental diets (LEb and HEb). Here again, survival and performance were compared and Ecophys.Fish was employed to help interpret observed results.

CHAPTER II

AUTOMATED CYCLING REGIMES OF TEMPERATURE AND DISSOLVED OXYGEN IN EXPERIMENTAL AQUARIA

SYNOPSIS

Scientists studying aquatic organisms often control abiotic factors such as temperature and dissolved oxygen, to better understand their biological impacts. Conventionally, studies involving the manipulation of these environmental factors are limited by cost or by technical expertise, reducing the treatments to constant or linearly changing regimes over time. Cyclical regimes, however, more accurately emulate the diel patterns of temperature and dissolved oxygen variation occurring in most natural aquatic ecosystems—and therefore could be more informative. Here I describe hardware and software components of an apparatus that was developed in response to the need for an autonomous system capable of inducing a cyclical regime of temperature and dissolved oxygen in experimental aquaria over an extended period of time. Using LabVIEW, a process-control program, the experimenter inputs the desired minimum and maximum values of temperature and dissolved oxygen as well as the hour of the day at which these values are to be attained. The system then generates a cyclical regime—in the form of a triangle wave—based on these inputs. Real-time data collected from an environmental monitoring probe within the experimental aquaria are interpreted by the software, and the appropriate pump or solenoid is activated to direct temperature and/or dissolved oxygen towards the target values. In practice, the range of the abiotic factors was restricted by the physical limitations of the system as well as the physiological

limits of the organism under study. Nevertheless, the technology represents the successful development of a highly-adaptable and relatively cost-effective means for autonomously inducing a cyclical regime of temperature and dissolved oxygen in experimental aquaria over extended periods of time.

INTRODUCTION

Historically, innumerable studies have attempted to assess the impacts of environmental factors on animal activity. Scientists working in the aquatic realm seem to have been particularly interested in abiotic factors, such as temperature and dissolved oxygen concentration (DO). A review of such studies is beyond the scope of this paper; however, the following list of citations indicates their persistence and variety: Fox and Simmonds 1932; Powers 1932; Fry 1947; Fry 1971; Zein-Eldin and Aldrich 1963; Weisepape et al. 1972; Neal 1975; Lakshmi et al. 1976; Brett 1979; Phares 1980; Neill 1990; Wedemeyer et al. 1990; Neill and Bryan 1991; Villarreal et al. 1994; Vargas-Albores et al. 1998; Buentello et al. 2000; Fontaine 2002; Neill et al. 2004; Chang et al. 2005; Fontaine 2007)

Typically, studies attempting resolution of biological responses to the manipulation of abiotic factors require close observation and meticulous care throughout the duration of long experiments. This is especially true when deviations in those environmental factors endanger the life of the organism and the integrity of the overall experiment. The costs of maintaining adequate manpower and logistical support clearly limit the scope of such experiments. Despite these challenges, experiments designed to evaluate effects of altered abiotic environmental factors are often desirable because of

their potential to expose complex interactions of environment and organism, or simply to extend the window of opportunity in which to study the organism under a particular set of conditions.

METHODS

In the 1980's, Plaia (1987) developed a computerized environmental monitoring and control system (CEMACS) to record and manipulate photoperiod and temperature in aquaculture systems. CEMACS represents a sophisticated application of once-modern technology and remains a relevant model for future automated control apparatus. Here, I describe the development and use of an updated CEMACS system. Mine was created in response to a need to study the effects of varying temperature and DO on juvenile red drum (*Sciaenops ocellatus*) performance. Thus, I designed a system capable of controlling and monitoring temperature and DO in experimental aquaria over time, with minimal intervention on behalf of the operator.

One of the environmental scenarios I required was that which emulates the diel cycle of temperature and DO typically occurring during the warm season in ponds and shallow bays of coastal Texas. In such situations, the early morning hours following sunrise are characterized by the lowest water temperatures due to overnight cooling and depleted DO because of high net respiration during the night. Conversely, water temperature and DO are at their maximum in the late-afternoon as a result of daytime heating and increased O_2 production from photosynthetic organisms, respectively.

Physical Description

Figure 2.1 illustrates the basic physical setup and information-flow of the environmental control apparatus. Functionally, the system was realized using a 1136-liter fiberglass-tank aquarium with independent, self-contained biofiltration. Two insulated 208 liter reservoirs served as the source of the temperature-treated water. The heated water reservoir was equipped with six 300-watt aquarium heaters (Tetra Acura Heaters) while the chilled water supply was provided by a 10,000 BTU/h chiller (Frigid Units, Inc. #D1-100, equipped with a Johnson Controls #A419 temperature controller). Water exchange between each temperature-treated water source and the main experimental aquarium was achieved via submerged powerheads (Maxi-Jet 1200). Dissolved oxygen was managed by altering flows of gaseous nitrogen and ambient air to multiple airstones distributed throughout the experimental aquarium. The nitrogen was provided by gasification of liquid nitrogen from a 160-liter, high-pressure tank; air was supplied via low-pressure regenerative electrical blowers. Electrical solenoids (#P442, DEMA Engineering Corporation) were used to control the type of gas being administered to the experimental aquarium.

A YSI 600R environmental monitoring probe (Yellow Springs Instruments, Yellow Springs, OH) was placed within the experimental aquarium for the duration of the experiment. At a rate of one reading per minute, the probe gathered and transmitted data on temperature (C), conductivity (mS/cm), salinity (ppt), DO (% saturation and ppm), and pH to an attached personal computer (Windows PC) equipped with LabVIEW software (National Instruments, Austin, TX).

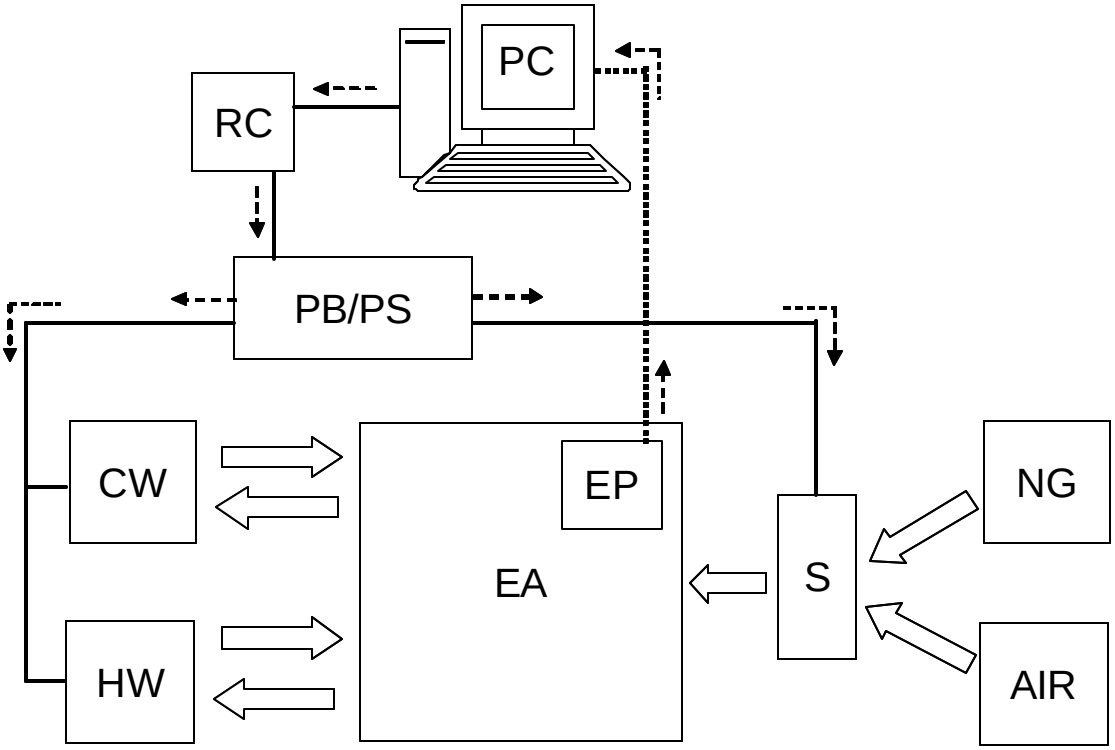

Figure 2.1. Schematic diagram of physical apparatus for monitoring and control of temperature and DO within the experimental aquaria (EA). PC, personal computer; RC, relay controller (ER-8); PB/PS, power box/power supply; CW, chilled water; HW, heated water; EP, environmental probe; S, solenoids; NG, nitrogen gas source; AIR, fresh air source. Dashed arrows indicate directions of flow for information and materials.

The computer was used to run EnviroControl, the LabVIEW virtual instrument (VI) system control software, which managed the external devices to induce the desired environmental regime (software description below). EnviroControl toggled power to 4 standard 110-volt electrical outlets on the power box via a relay control device (ER-8; National Instruments). Using power generated via an external power supply (#XP581, Elenco Power Supply), the ER-8 activated or deactivated solid-state relays to energize the outlets as necessary. Both powerheads and solenoids were plugged into the computer-controlled outlets, thus providing control of water temperature and DO, respectively, within the experimental tank. Two submerged powerheads were used to encourage mixing of treated water within the experimental aquarium.

Software Description

The EnviroControl program was developed using the LabVIEW programming environment (National Instruments, 2000). LabVIEW is a powerful graphics-based programming language used to develop user-friendly software applications capable of joining data acquisition devices with physical control interfaces. Regrettably, the high purchase price of the full LabVIEW software suite may be beyond the range of many budgets. Therefore, a software-independent mathematical description of the generalized algorithm is provided in the Appendix to facilitate conversion into alternative programming languages. The exact programming used is highly dependent on the end-user's particular hardware and software setup. Because the algorithm is customizable for a wide range of hardware, the important details included here will be limited to those inputs and functions critical to the triangle-wave algorithm.

The triangle-wave algorithm is used to calculate target values of temperature (C) and DO (ppm)—independent of each other—based on user inputs. The user sets the minimum and maximum value of each factor (E_{min} and E_{max}, respectively) and the hour of the day at which these values are to be achieved (T_{min} and T_{max}, respectively). Via a linear function, these inputs are used to generate the period and frequency of a symmetrical triangle, or saw-tooth, wave which provides the "target values" of temperature and dissolved oxygen for the particular time of the day. In this case, the time-step utilized—one reading per minute—allowed adequate control of both temperature and DO while archiving ample data on relevant input and output variables.

Once activated, EnviroControl executes the triangle-wave algorithm at every time-step. It detects the current value of each environmental factor and compares it to the calculated target value by interpreting the direction of deviation. EnviroControl then transmits a signal to the relay control device to energize the appropriate electrical outlet and thus direct the environmental factor towards its target value. To control temperature, submerged powerhead pumps exchange either heated- or chilled-water with the experimental aquarium while solenoids toggle between nitrogen gas and air to manage the level of dissolved oxygen as appropriate.

The front panel and the visual- and text-based outputs (Figure 2.2) were designed to assist the user in readily identifying the present-state of the environmental factors as measured by the probe, as well as facilitating data manipulation and analysis of archived data. In addition to basic diagnostic displays, lights on the panel give a clear visual indication of the power status of each external control interface. As well, the current readings from the probe and both a graphical and a scrollable history of temperature and DO are displayed. Upon initialization of the program, a file-header containing a date- and time-stamp and column labels is created on a text file. At each subsequent time-step, EnviroControl outputs to this text file the data string as output from the probe as well as the user's input parameters and the calculated target values for that iteration.

RESULTS AND DISCUSSION

I utilized the EnviroControl apparatus to create a cyclical regime of temperature and DO for a series of experiments that ran continuously for 27 – 36 days. Figure 2.3 shows a sample excerpt of data from the first 72 hours of one of these experiments. Temperature, DO, and salinity were monitored via a handheld environmental monitoring probe (YSI 85) at least twice daily throughout each experiment. To ensure consistency, these readings were compared to current values as measured by the main environmental monitoring probe (YSI 600R), and they were verified against the target values as calculated by EnviroControl; adjustments to the system then were made as necessary.

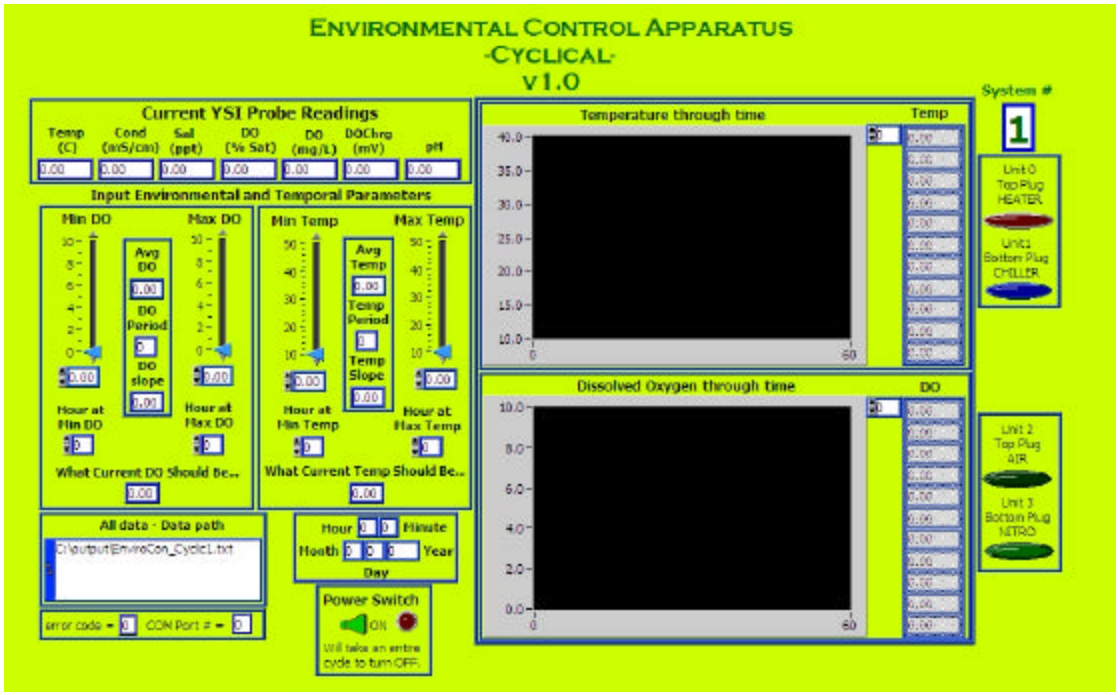

Figure 2.2. Front panel (user interface) of the cyclical version of the EnviroControl system prior to setup and initialization by user. See text for further details regarding the various inputs and outputs.

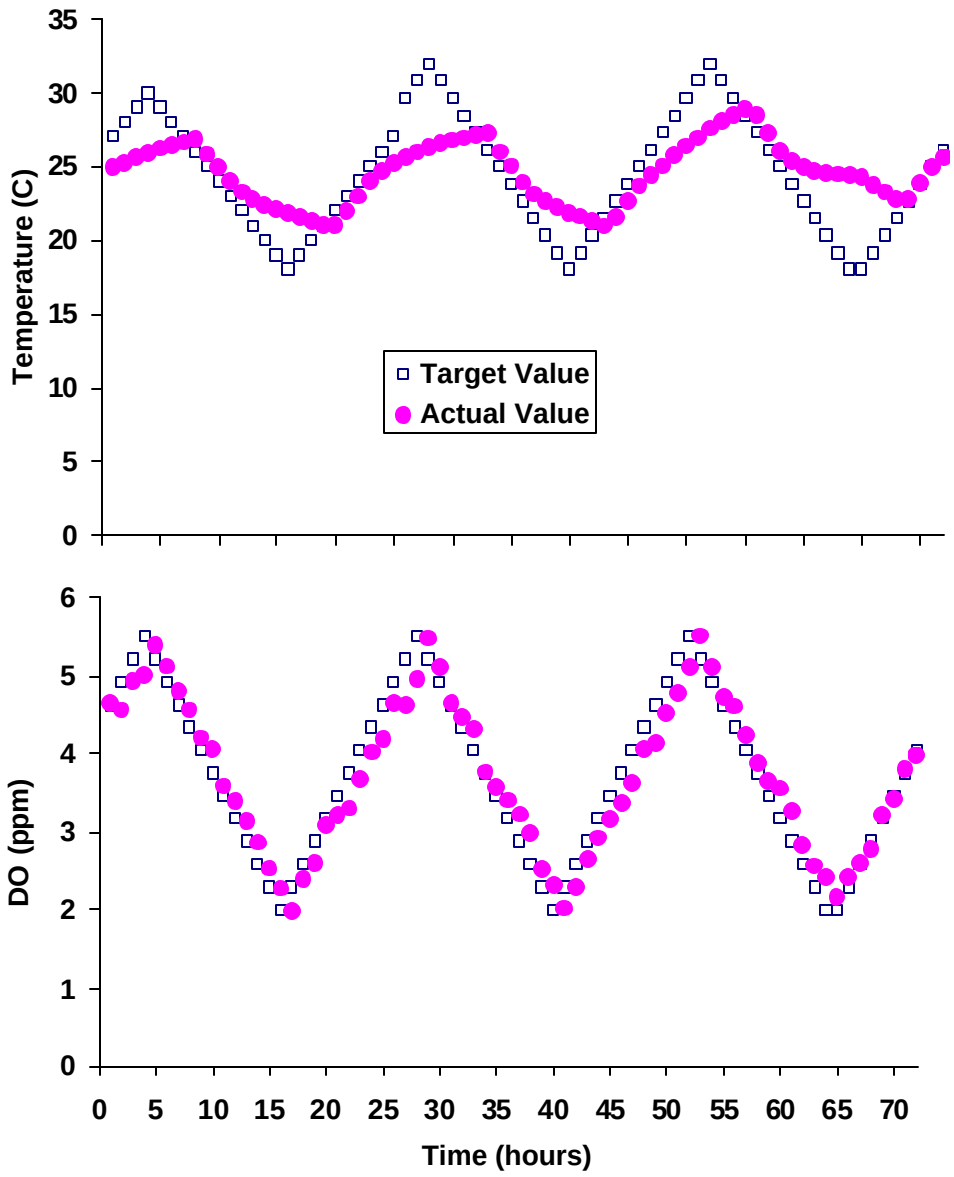

Figure 2.3. A 72-h sample of actual values observed—as recorded by the environmental monitoring probe—and target values—as calculated by the triangle-wave algorithm—for both temperature (C) and DO (ppm). The EnviroControl system recorded data on a per-minute basis; the data shown here have been filtered to show hourly readings only.

In practice, I quickly and easily induced a range of DO of up to 5.6 ppm, from 0.8 – 6.4 ppm. The actual pattern utilized in each experiment was dependent on the particular requirements of that study. Each 160-liter tank of liquid nitrogen lasted 5 – 10 days, depending on such factors as the amplitude of the desired DO cycle, the temperature regime, the condition of the valves and other hardware on an individual tank. I employed a 2-stage inert-gas regulator (#VTS 250-B-580, Victor Equipment Company) in order to maintain a consistent outflow of nitrogen gas despite the decrease in pressure within the tank over time. Nevertheless, adjustments of flow-rate for both the nitrogen and air sources were necessary upon exchanging an exhausted tank with a fresh tank.

I originally sought to induce a cyclical pattern of temperature ranging approximately 10 degrees, from 18 – 28 C. In practice, however, I was only able to induce a maximum range of 8.6 degrees, from 19.5 – 28.1 C. As with DO, the amplitude of the temperature cycle achieved was dependent on the particular experiment. Additionally, thermal inertia of the water mass caused its temperature to exhibit hysteresis such that the system was unable to achieve the target value at the target time. By exaggerating the range of temperature extremes input into EnviroControl, however, I was able to optimize the temperature cycle within the experimental aquarium to better represent the desired pattern. In retrospect, the volumes of the heating and chilling reservoirs were too small relative to that of the experimental tank.

In addition to the physical limitations described above, various technological issues proved to be challenging as well. A lack of computer hardware—RS-232 ports,

specifically—limited the number of environmental monitoring probes that could be interfaced with EnviroControl. As well, the relay control device and power box were limited to eight relays and outlets, respectively. These limitations, however, could be resolved by the acquisition or development of the appropriate hardware and software.

This version of EnviroControl was designed to interface with YSI 600R environmental monitoring probes. These probes are highly adaptable for collection of data on various environmental parameters at user-defined intervals and they proved to be generally reliable with the proper maintenance. Their initial cost and cost of upkeep can be restrictive to some budgets, however. If a user desired to collect data on temperature and DO only, other environmental monitoring solutions are available. Despite the seemingly high cost to acquire all the hardware and software used in this study, it was our experience that commercially-available systems would have been even more expensive and unable to perform as desired.

While not presented in detail here, an alternate version of EnviroControl was developed. During testing, EnviroControl.Constant was able to successfully maintain environmental factors—again, temperature and DO—within a user-defined range. The user is able to restrict the target values of environment to the desired level by setting the minimum and maximum inputs to a narrow range. For example, a minimum of 24 C and maximum of 26 C would ensure that temperature remained at 25 C +/- 1 C during the course of the experiment. The constant version of EnviroControl lacked the triangle wave algorithm; otherwise, both versions functioned similarly. Due to the lack of

computing resources, however, only the cyclical version of the program was employed for my experiments.

Our EnviroControl Apparatus successfully controlled both temperature and DO within the physical limits of the system with minimal interference on behalf of the user. In general, DO was easier than temperature to manipulate because of the ability to rapidly saturate the experimental aquaria with large amounts of air or nitrogen as necessary; whereas, temperature was restricted to a more narrow range than originally desired. Nevertheless, I feel that the technologies developed and described here could be further refined and subsequently employed by those who desire to impose a variety of environmental regimes upon aquatic organisms.

CHAPTER III

EFFECTS OF TEMPERATURE AND FEED ENERGY ON PERFORMANCE OF JUVENILE RED DRUM*

SYNOPSIS

We tested the hypothesis that the growth of fish exposed to high temperatures can be limited by available food energy whereas that of fish exposed to low temperatures can be limited by their metabolic capacity to exploit the available food energy. Under laboratory conditions we evaluated growth (%/day) and marginal metabolic scope MMS; $L \cdot g^{-1} \cdot h^{-1}$) of juvenile red drum Sciaenops ocellatus exposed to two levels of dietary energy, low (LE; ~ 4.1 kJ/g) and high (HE; ~ 15.9 kJ/g), and to three temperatures, approximately ~ 19, ~ 25, and ~ 29°C, for a period of 6 weeks. Growth rate and MMS increased with temperature, but only growth rate increased with dietary energy and then only at the higher two temperatures. The simulation model Ecophys.Fish was employed to elucidate experimental results potentially confounded by interactions between fish weight and the controlling effects of temperature on metabolism. The simulated and observed results both showed that performance is enhanced at higher temperatures, especially for fish consuming the HE diet. A subsequent 6-week-long experiment confirmed results for fish fed the two diets at ambient temperature (~26°C) and sought to

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*Reprinted from Transactions of the American Fisheries Society, Vol.136. Fontaine, L. P., K. W. Whiteman, P. Li, G. S. Burr., K. A. Webb, J. Goff, D. M. Gatlin III, W. H., Neill, K. B. Davis, and R. R. Vega. Effects of temperature and feed energy on performance of juvenile red drum, pp. 1193-1205. Copyright 2007, with permission from the American Fisheries Society.

further resolve responses by examining body condition indices and proximate composition. Additionally, these fish were assayed for differential cortisol response to 15 min of confinement stress. The feed efficiency, hepatosomatic index, intraperitoneal fat ratio, and whole-body fat of fish fed the LE diet were significantly lower than those of fish fed the HE diet, indicating relative energy malnutrition in the LE group. As with MMS, no apparent differential effect of feed energy on the pre- or poststress values of plasma cortisol was observed. These findings support the ideas that red drum obtain greater metabolic capacity when they are exposed to a near optimal temperature and that their ability to transform that capacity into growth is maximized only when they are provided a nutritious, high-energy diet.

INTRODUCTION

The red drum *Sciaenops ocellatus* is a valuable seafood and recreational-fishery resource throughout its natural range, which extends from the middle-Atlantic coast of the US, through the Gulf of Mexico, and into the Caribbean Sea (Matlock 1990). Over-harvest and consequent decline of the red drum stock in the Gulf of Mexico prompted Texas and other Gulf-coast states to prohibit sale of wild-caught red drum after 1981, and motivated hatchery production of red drum juveniles at state-operated facilities in Texas for stock enhancement (McEachron et al. 1995; Vega et al. 1995; McEachron et al. 1998; Blaxter 2000). Red drum are now cultured as food-fish in Texas and elsewhere, both in the US and abroad (Chamberlain et al. 1990; Diamant 1998; Gatlin 2000; Lee and Ostrowski 2001; Hong and Zhang 2003).

In the wild, juvenile red drum inhabit environmentally-variable coastal ecosystems and forage upon a variety of invertebrates and small fishes (Simmons and Breuer 1962). The conditions associated with artificial rearing, on the other hand, are often manipulated to provide optimum environmental and bioenergetic situations for growth and survival (Luebke and Strawn 1973; Neill 1990; Gatlin 2002a).

Consideration of interacting environmental and bioenergetic factors has led to the development of a computer-based ecophysiological model, Ecophys Fish, which can simulate effects of time-varying environment and feed properties on metabolism and growth of red drum (Neill et al. 2004). Ecophys.Fish provides a platform from which researchers and fisheries managers alike can explore "what if" scenarios by altering the inputs of environment (e.g., temperature, salinity and dissolved oxygen) and feed energy-density and digestibility, to examine the potential impacts of their interactions on red drum performance. Additionally, Ecophys.Fish can aid in the interpretation of complex interactions of biology and environment via the parameter MMSO—the residual intercept of marginal metabolic scope; by accounting for interacting effects of total environment and fish status, MMSO serves to capture the inherent metabolic efficiency of the fish-environment system (Neill and Bryan 1991; Neill et al. 2004). A second parameter, the Winberg-adjustment, allows accommodation for the variable fraction of metabolic scope the fish may use for routine metabolism beyond that used for standard metabolism (Winberg 1960; Neill et al. 2004). Together, MMSO and the Winberg-adjustment provide a convenient framework within which experimental results can be resolved and interpreted.

The purpose of this paper is to report results of two laboratory experiments conducted to test and resolve hypothesized responses emerging from Ecophys.Fish. In particular, we tested the hypothesis that feed energy-density can be limiting to performance of red drum juveniles at high temperature, but that it is metabolic capacity which tends to limit performance at lower temperature. The first of the two experiments focused on the interactive effects of temperature and dietary energy-density on red drum growth and metabolism; the second sought to confirm the striking contrast in performance of red drum fed low- and high-energy feeds at higher temperatures, and to resolve performance differences in terms of proximate body composition and cortisol response to handling stress.

METHODS

Preparation of Experimental Diets

Two experimental diets with contrasting energy densities—low energy and high energy—were utilized to test the proposed hypotheses. Because natural forage of red drum typically contains high levels of moisture and low levels of energy, relative to prepared feeds, water with 2% agar was used to maintain the moisture content and dilute the energy in the "low-energy" (LE) diet. The protein requirement of the carnivorous red drum was met by incorporation of menhaden fish meal and fish solubles (Omega Protein, Inc., Houston, TX). Fish meal provided 72.4% of the crude protein. Fish solubles were used mainly to increase the liquidity of the mixed ingredients and facilitate their uniform distribution. The LE diet was formulated to contain 12% protein, 2% lipid and 3.35 kJ/g digestible energy on a fresh-weight basis (Table 3.1).

Table 3.1. Composition of experimental diets. Values down to and including "D.I. Water" are percentages of diet weight as-manufactured; values for "Moisture," "Protein," "Lipid," and "Ash" are percentages of weight as-fed; values for "Gross Energy" are joules/gram as-fed.

Constituent	Diet	
	LE	HE
Menhaden fishmeal[a]	15.00	57.50
Fish soluble [a]	9.00	4.00
Dextrin [b]	--	2.00
Menhaden oil [a]	--	4.07
Vitamin premix [c]	0.10	3.00
Mineral premix [c]	0.10	4.00
Carboxymethyl cellulose [b]	--	2.00
Celufil [b]	0.80	4.40
Agar [b]	2.00	--
DI Water	73.00	--
Moisture	5.3	13.1
Protein	12.7	34.8
Lipid	2.7	8.5
Ash	5.1	13.0
Gross Energy	4100	15900

[a] Omega Protein Corporation, Houston, TX, USA. Menhanden fish meal (Special Select ™) contained 69.5% protein and 10.3% lipid on a dry-weight basis.
[b] US Biochemical Corp., Cleveland, OH.
[c] Same as Li et al. (2004).

Development of the forage-simulating, low-energy diet proved non-trivial; thus, methods of preparation are provided here in some detail: After DI water was heated to a boil, agar was added and stirred-in thoroughly. All the dry ingredients then were added to the agar-water mixture; finally, fish solubles were added with vigorous stirring until the slurry appeared homogeneous. The slurry then was poured and spread evenly into a tray, to just fill a contained plastic grid made of 1-cm-square "egg-crate louver" (of the type manufactured as a light-diffuser for fluorescent lighting fixtures); the tray containing the diet-packed grid, and with its horizontal orientation maintained, was immediately placed into a freezer for rapid solidification of the diet without obvious precipitation. After chilling (but not freezing), the solidified diet was removed from the freezer, shaken and knocked from the grid, and the cubes chopped and crumbled as necessary to match the gape of the fish to be fed. The low-energy diets then were stored in sealed bags in a refrigerator at 4°C until fed. Consistency of the low-energy diet as fed was similar to that of "gritty" dessert gelatin.

The high-energy (HE) diet (Li et al. 2005) was formulated to contain 40% protein, 10% lipid and 15.9 kJ/g digestible energy on a dry-weight basis. This formulation meets or exceeds all known nutritional requirements of red drum and most warmwater fishes (NRC 1993; Gatlin 2002b; Li et al. 2005). The experimental HE diet was processed following the procedures described by Webb and Gatlin (2003) and was stored in sealed bags in a freezer at -20°C until thawed and fed. Consistency of the high-energy diet as fed was that typical of pelleted diets like those used in trout and salmon production.

Crude protein in both the low-energy and high-energy diets was assayed with a Leco® Protein Analyzer (Leco Corporation, St. Joseph, MI, USA) by the Dumas method (AOAC 1990) after the diet samples were dried at 135°C for 2 h, and calculated as percent nitrogen multiplied by 6.25. Analysis of moisture, ash and lipid followed established procedures (Webb and Gatlin 2003).

Bomb calorimetry estimated as-fed energy densities of the LE and HE diets to be approximately 4.1 kJ/g and 15.9 kJ/g, respectively (Table 3.1). A digestibility trial, following the procedures described by Gaylord and Gatlin (1996), estimated energy digestibilities of the LE and HE diets to be 74.8 % and 72.9 %, respectively, for 300 – 350 g red drum at a temperature of 29 ^{o}C and a salinity of 5 ppt (Fontaine, et al., unpublished data).

Experiment I. Effects of Temperature and Feed Energy-Density on Performance of Juvenile Red Drum

Earlier work with Ecophys.Fish led to the development of what we consider an energy/metabolism-tradeoff hypothesis, which proposes that fish exposed to higher temperatures may become limited by the amount of food-energy they are physically able to consume and physiologically process, even under conditions of unlimited food availability, if that food has low energy-density; whereas, fish exposed to lower temperatures tend not to be limited by low energy-density of food, but by their low metabolic scope for growth. Such an hypothesis is not entirely novel: McLaren (1963) for zooplankton and later Brett (1971) for salmonids suggested that diel vertical migration in thermally stratified lakes might confer an “energy bonus” that could be

exploited for greater growth. The energy-bonus hypothesis relied on exposure to low temperatures to reduce various temperature-dependent physiological costs, thus increasing discretionary energy available for growth (McLaren 1963; Brett 1971). In contrast to McLaren's idea, the hypothesis of energy/metabolism tradeoff suggests that it is the controlling effect of elevated temperature on metabolism (Fry 1947) that enables greater growth--but only if food is ample, nutritionally adequate, and energy dense (and that no other environmental factor is limiting). Under the simulation model Ecophys.Fish, diel regimes of cyclic temperature and dissolved oxygen (DO), coupled with energy-rich food, may confer an "energy bonus" leading to large growth rates—but the causal increase in metabolic scope for growth owes not to a decrease in routine metabolism at low temperature, but rather to an increase in active metabolic rate (metabolic capacity) during the high temperature-DO phase of the cycle (Neill et al. 2004).

In Experiment I, to test the energy/metabolism-tradeoff hypothesis, we fed individual red drum the LE or HE diets under prescribed temperature regimes for a period of 6 weeks. Responses were evaluated as growth rate over the experiment, and as capacity for metabolic performance estimated in terminal respirometric assays.

Fish and Feeding

Juvenile red drum (*Sciaenops ocellatus*) were obtained from Texas Parks and Wildlife Department's Perry R. Bass Marine Fisheries Research Center, located in Palacios, TX. The fish were transported to Texas A&M University System's Aquacultural Research and Teaching Facility (ARTF), near Snook (Burleson Co.), TX,

where they were subjected to a 3-week conditioning period in a brackish-water (10 ppt), recirculating system prior to the experiment. A commercial maintenance diet was fed to all the fish during the first 2-wk of the conditioning period, after which fish averaging approximately 0.4 g were graded by size and stocked as groups of 5 individuals into each of 12 cages within each of six 1,136-liter, circular fiberglass-tanks. Thereafter, each cage within a tank was randomly assigned a dietary treatment (6 cages LE; 6 cages HE) and received this ration throughout the remainder of the experiment. The structural design and layout of the cages within a tank (see cage description below: mesh screen, feeding tube, false-bottom, etc.) prevented transfer of experimental diets between cages. After one additional week, initial weights of fish were obtained, including those of "spares" added to cages to replace dead or missing—presumably cannibalized—individuals; spare fish had been maintained on the same dietary regime as that of individuals they replaced. Going forward into the experiment, the initial weight of individual fish across all cages was 2.1 ± 0.57 g (mean ± SD).

The 12 cages were distributed as evenly as practical, between the outer wall and the central biofilter in each circular tank. Each cage consisted of a submerged 19-liter (5 gallon) polyethylene bucket with lid; ~16-cm diameter disks of plastic had been removed from the center of each bucket's bottom and lid, and replaced with 0.5-cm plastic mesh, to allow for exchange of water between cage and tank. Low-pressure electrical blowers provided aeration via air stones both inside and outside the buckets, to facilitate water circulation and to maintain dissolved oxygen (DO) levels near air-saturation. Resting inside and near the bottom of every cage, an air stone encouraged upwelling, pumping

water into the cage through the bottom mesh and out through the top mesh. Water circulation was further facilitated by raising the cages ~6 cm off the tank's bottom, on a false bottom of plastic "egg-crate louver" supported by short lengths of PVC pipe.

Water flow-rate through each biofilter was maintained at approximately 1 tank-volume per hour via an aquarium powerhead. Water quality was deemed adequate throughout the experiment, in that total ammonia nitrogen did not exceed 0. 6 mg/L. Uneaten food and expelled feces were siphoned from the tanks on a weekly basis. Cages were similarly cleaned at each fish-weighing event. Salinity was maintained at 10-12 ppt by adding synthetic sea salt (Fritz Industries Inc., Dallas, TX) to freshwater from an on-site well. After each weighing event ~30 % of the water in each experimental tank was removed by siphoning and draining, then replaced with new water. A 12 h light: 12 h dark photoperiod was maintained with overhead fluorescent lights controlled by timers.

The "ambient" water-temperature treatment (~25 °C) was maintained by regulation of the air temperature in the laboratory. The "cool" temperature treatment (~19 °C) was achieved by thermostatically controlled electric chillers and the "warm" temperature treatment (~28 °C) by thermostatically controlled electric aquarium heaters.

The three experimental temperature regimes were randomly assigned to pairs of tanks; the members of each pair were intended to be duplicates in that each experimental diet (low-energy = LE and high-energy = HE) was fed to 6 replicate cages within each tank. Temperature variation between duplicate tanks was minimal: Cool-1 = 18.8 ± 0.27 ^{o}C; Cool-2 = 19.0 ± 0.31 ^{o}C; Ambient-1 = 24.7 ± 0.34 ^{o}C; Ambient-2 = 25.1 ± 0.34 ^{o}C;

Warm-1 = 27.9 ± 0.10 ^{o}C; Warm-2 = 27.9 ± 0.13 ^{o}C. Fish were fed twice daily for the duration of the experiment. On a weekly basis, environmental data (temperature, salinity, dissolved oxygen, and pH) and the mean weight of the fish in each cage were input into Ecophys.Fish and used to simulate feed consumption; this simulated amount then was increased substantially, to ensure that fish were fed in excess of the amount they could actually consume and to account for feed not eaten and potentially lost through the bottom of a cage. Feed was administered via a plastic feeding tube, which protruded above the water and ensured that feed was deposited into the cage for which it was intended. Survival and growth were monitored bi-weekly, by removing, counting and individually weighing the surviving fish from each cage.

Due to inconsistently varying rates of mortality caused, we believe, by agonistic interactions, the fish surviving in experimental cages after 4 weeks ranged from 1 to 5 individuals. In an effort to rebalance the design and reduce unintended density-dependent effects (i.e., social interactions depending on the number of remaining fish and their variation in size) on performance (Kendal et al. 2004), all individuals except the largest were culled from each cage. Comparability of growth data before and after the culling operation was sought by restricting the growth analysis in Experiment I to the largest fish in each cage, throughout the experiment. We assumed that the fish within a cage did not change their rank by size, and that the largest fish at the initial weighing was the last survivor. Additionally, for the subsequent statistical analysis and modeling effort, responses were based on the mean of replicate fish receiving the same dietary-treatment within a given temperature-treatment.

Following the methods of Springer and Neill (1988) and Neill and Bryan (1991), automated routine respirometry was performed, to estimate metabolic capacity of red drum after 40 days exposure to the experimental treatments. Respirometry was conducted on individual fish with the respirometric chambers submerged in the experimental tanks in which the fish had been kept, to ensure consistency and continuity of temperature and water-chemistry regimes. In order to measure routine metabolic rate and not standard metabolic rate, the 12:12 light regime was supplemented with continuous, low-intensity fluorescent lighting for those fish undergoing respirometric assays; additionally, this ensured a uniformity of light-levels across and within experiments, also considered potentially important. Fish were fasted for 24 h and weighed just prior to placement into the chambers. After ~ 20 h of respirometric measurements, fish were removed from the chambers, euthanized, and frozen for storage (-20°C). Immediately following each "fish run," the net oxygen uptake by other system components (biological and chemical oxygen demand; BCOD; $mgO_2 \cdot g^{-1} \cdot h^{-1}$) for that trial was estimated with a 1-h "blank run," with the corresponding empty respirometer chamber. We obtained for each fish a median value of routine metabolic rate, RMR (adjusted for BCOD; $mgO_2 \cdot g^{-1} \cdot h^{-1}$); limiting oxygen concentration (LOCr; the level of dissolved oxygen at which point routine metabolism becomes limited; mg/L); and, their ratio, marginal metabolic scope = MMS = RMR/LOCr. Generally speaking, a larger MMS ($L \cdot g^{-1} \cdot h^{-1}$) value indicates a greater capacity for physiological performance under conditions of the test environment (Neill and Bryan 1991; Neill et al. 2004). MMS was calculated for each fish as the median of the ratios formed by the time series of RMR

and LOCr pairs obtained during periods in which fish and apparatus were least likely to be disturbed by personnel (Neill and Bryan 1991; Springer and Neill 1988). Prior studies employing similar respirometric assays on individual fish have shown that restricting data collection to this time period, in combination with selecting the median values of metabolic response, provides a more consistent measure of the response by reducing variation not attributable to experimental treatments (Fontaine 2002; Clark 2003; Vega 2003; Neill et al. 2004). Respirometry trials typically yielded at least 4 acceptable pairs of RMR and LOCr values per fish.

Statistical Analysis and Modeling

Growth performance and metabolism data were subjected to analysis of variance with SPSS to test for effects of temperature and feed energy on the treatment mean response of the six replicate fish in each large tank. While pooling the replicates in this manner lowers statistical power, it minimizes concerns over pseudoreplication—while preserving the integrity of the data— by more conservatively reflecting the levels of response that were presented by the fish. Differences were considered significant at $P < 0.05$.

The ecophysiological model—Ecophys.Fish—was used to simulate growth and metabolic performance of red drum from Experiment I. The mean environment (temperature, DO, salinity, pH on a 1-h time-step) of the replicate treatment tanks was processed through the model to emerge as growth rate, MMS, and RMR (Neill et al. 2004). Consistent with the statistical analysis, comparisons of growth and metabolic data were based on the treatment mean response. We assumed that 1) modeled fish were

not limited by rate of feed presentation (unlimited FeedRate); 2) energy density of feed (GEfeed) was 4.10 kJ/g for the low energy diet and 15.90 kJ/g for the high energy diet, with corresponding energy digestibilities (FeedDigestibility%) of 74.8 % and 72.9 %, respectively; and, 3) energy density of modeled fish (GEfish) was initially 4.18 kJ/g (natural weight) but varied thereafter as a function of energy intake relative to the cost of routine metabolism. The latter assumption was implemented by having GEfish increase at 0.3%/day to a maximum of 5.86 kJ/g when the cost of feed-processing metabolism (Ad) exceeded 60 % of routine metabolic rate (Ar), and decrease at 0.3 %/day to a minimum of 3.35 kJ/g when the cost of feed-processing metabolism fell below 60 % of routine metabolic rate; this assumption is different from that of Neill et al. (2004) only in that the 60 % value here replaces their ("rather arbitrary") 20 % value. The change from 20 % to 60 % is justified below, in the context of our experimental (not modeling) results. (Note: Acronyms used by Neill et al. (2004) are provided here to assist the interested reader in comparing our modeling conventions and results with theirs (Neill et al. 2004; also, see http://neilllab2.tamu.edu/EcophysFish/EcophysFish.htm). Iterative simulation proceeded with manipulation only of the MMSO and the Winberg-adjustment parameters until an optimum match between observed and simulated pairs of RMR, MMS, and growth rate were achieved. A match was considered optimum only when the coefficient of determination was greater than 70 % for RMR, MMS, and growth rate, simultaneously.

Experiment II. Confirmation and Resolution of Feed Energy-Density Effects on Performance of Juvenile Red Drum

The differences in growth rates of juvenile red drum fed daily to satiation on the LE and HE diets at ambient and higher temperatures in Experiment I were so striking that we sought confirmation and further resolution in a second experiment, an experiment performed with larger groups of fish and under conditions more typical of the conventional laboratory feeding trial. We compared effects of the LE and HE diets fed under ambient-temperature conditions, on red drum growth, marginal metabolic scope, and several other performance measures. These additional performance measures included body-condition indices and proximate composition, as well as cortisol response measured prior to and after a confinement-stress test. Body condition and proximate composition are highly informative indicators of nutritional adequacy (Craig et al. 1995, 1999; Lovell 1998). Elevated blood-titers of the adrenal hormone cortisol are indicative of stress, and in teleosts typically are associated with depressed growth, metabolism and immunocompetence (Barton and Iwama 1991; Van Weerd and Komen 1998; Pérez-Domínguez and Holt 2006).

Fish and Feeding

This experiment involved groups of 20 individual red drum maintained in 110-L aquaria in a closed recirculating system at ~26°C. Here, dietary energy-density was the primary experimental factor under evaluation. Growth and associated bioenergetic responses, metabolic performance, and cortisol response to handling stress were measured for fish fed the same LE and HE diets as in Experiment I.

Juvenile red drum were obtained from the Marine Development Center, operated by Texas Parks and Wildlife Department in Corpus Christi, TX, and were subjected to a 2-wk conditioning period at Texas A&M University System's ARTF in a brackish-water (8 ppt), recirculating system prior to the feeding trial. The HE diet was fed to all the fish during the 2-wk conditioning period, after which fish averaging approximately 1 g then were graded by size and stocked into 110-L aquaria as groups of 20 individuals having a total weight per group of 21.1 ± 0.5 g (mean ± SD). Water flow-rate was kept at approximately 650 mL/min per aquarium via a recirculating system that maintained adequate water quality (total ammonia nitrogen = 0. 6 mg/L) through biological and mechanical filtration. Salinity was maintained at 7-8 ppt by adding synthetic sea salt (Fritz Industries Inc., Dallas, TX) to freshwater from an on-site well. Low pressure electrical blowers provided aeration via air stones and maintained DO levels near air-saturation. Water temperature was controlled by regulation of air temperature in the laboratory and remained at 26 ± 1°C throughout the trial. A 12 h light: 12 h dark photoperiod was maintained with fluorescent lights controlled by timers.

Each experimental diet was fed to three replicate aquaria of fish for 6 wk. All groups were fed their respective diets at the same fixed rate on a dry-weight basis, starting at 7% of body weight per day for the first 3 wk and then 6% for the last 3 wk. These rates were based on previous studies using red drum and were designed to assure satiation without the wastage of feed and consequent environmental degradation commonly associated with overfeeding (Moon and Gatlin 1994; Gaylord and Gatlin 1996; Webb and Gatlin 2003; Li et al. 2005). Fish were fed at 0830 and 1730h, 7 d each

wk. Growth and feed efficiency were monitored weekly by collectively removing and weighing the fish from each aquarium.

Sample Collection and Confinement-Stress Test

At the end of the feeding trial, three representative fish from each aquarium were obtained randomly and, from each fish, approximately 0.4 mL of blood was collected from the caudal vasculature using a 1-mL heparinized syringe and 27-gauge needle. Blood collection occurred, as recommended by Davis and Griffin (2004), within a 2-min period and without the use of anesthetics, to estimate pre-stress cortisol levels. Serum from each fish was separated after centrifugation and was stored at -80°C until shipped on dry ice to the Harry K. Dupree Stuttgart National Aquaculture Research Center for cortisol analysis (Li et al. 2005). From these pre-stress fish, hepatosomatic index (HSI = liver weight × 100/body weight) and intraperitoneal fat (IPF) ratio (weight of IPF × 100/body weight) were determined for each individual; then, the three fish were pooled, homogenized and samples subjected to proximate analyses to estimate fractions of whole-body mass contributed by moisture, protein, lipid and ash, according to established procedures (Webb and Gatlin 2003).

Another three fish from the same aquarium were obtained randomly and placed in a container (30 cm × 25 cm × 12 cm) with 0.4 L of water from the culture system for 15 min, to impose a controlled confinement stress. After the confinement period, post-stress fish were bled and the serum obtained, stored, and shipped as previously described for the pre-stress group.

Growth and Metabolic Performance

Subsequent to the first phase of the feeding trial, those fish not used for non-growth-related analyses continued to receive the treatment diet without interruption. This subset of fish was used to confirm the growth and metabolic results obtained in Experiment I. Respirometric assays of individual fish began 48 days from the start of experimental feeding. Procedures for the metabolic assay were as described above for Experiment I.

Statistical Analysis

Data on body-condition indices, body composition, and pre-stress cortisol were based on the responses of three fish in each of three replicate tanks and were subjected to analysis of variance. Post-stress serum cortisol data were subjected to the non-parametric Mann-Whitney test because of unequal error variances across the post-stress groups. Pre- and post-confinement stress data were subjected to Student's *t* test. Data on the other performance measures--feed efficiency, survival, growth rate, LOCr, RMR, and MMS--were evaluated via analysis of variance. For these data, the median response of an individual fish during the period of least personnel disturbance from each of the three replicate tanks served as the basic statistical unit. As before, analyses were performed using SPSS with differences considered significant at $P < 0.05$.

RESULTS

Experiment I

Growth performance was evaluated by calculating relative growth rate (%/day) as the percent change in weight per day using initial weight of the largest fish at

stocking, final weight of the largest fish, and the number of days the fish had spent in its cage.

Growth rate increased with temperature (df = 11; $P = 0.000$), but much more so for the HE treatment than for the LE treatment (Figure 3.1). Growth rate varied from 7.1 to 18.9 %/day, and analysis of variance indicated that feed energy, temperature, and their interaction all were significant. The mean percentage weight increase of fish consuming the LE diet (10.0 %/day) was significantly lower (df = 11; $P = 0.002$) than that of fish consuming the HE diet (14.1 %/day). Post-hoc testing revealed that growth rate of fish from the cool-temperature treatment (7.4 %/day) was lower than that of fish from the ambient- and warm-temperature treatments (13.1 and 15.6 %/day, respectively); however, no significant difference was detected between growth rates of fish in ambient- and warm-temperature treatments ($P = 0.117$). A significant result for the test of interaction between feed energy and temperature (df = 11; $P = 0.038$) indicated that the relative growth rates of fish consuming feeds at the two levels of energy differed among the various temperature treatments. Larger differences in growth rate were observed between dietary energy treatments at the higher levels of temperature. Specifically, in cool water the growth rate of fish consuming LE diet was 7.1 %/day versus 7.6 %/day for those on the HE diet; whereas, fish consuming the high-energy diet in ambient- and warm-temperature treatments (15.7 and 18.9 %/day, respectively) greatly out-grew their counterparts on the low-energy alternative (ambient, 10.5 %/day; warm, 12.2 %/day).

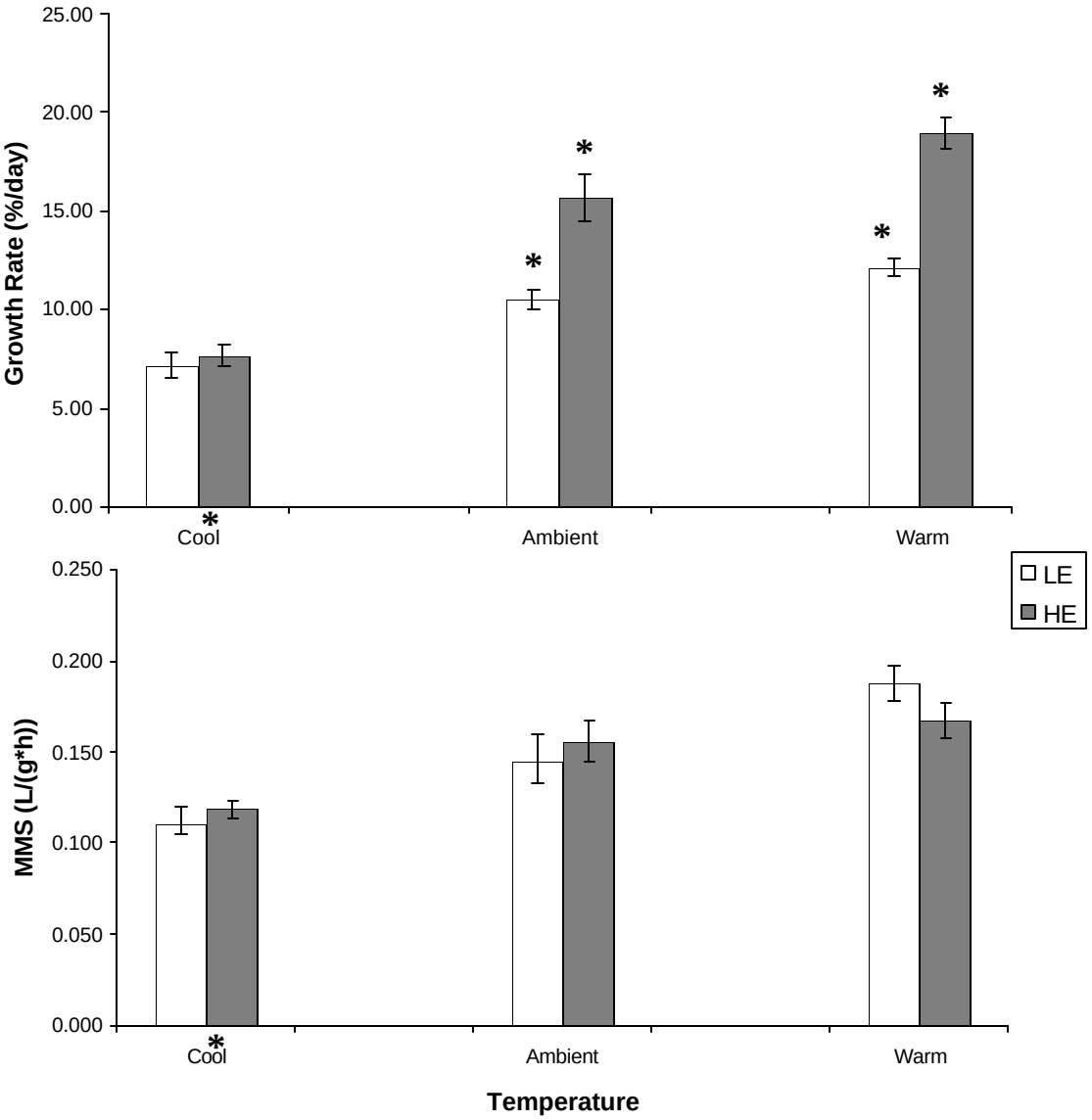

Figure 3.1. Growth rate (%/day) and marginal metabolic scope (MMS; $L \cdot g^{-1} \cdot h^{-1}$) for red drum consuming either a low energy diet (LE) or a high energy diet (HE) and exposed to three levels of temperature (Cool ~19°C; Ambient ~ 25°C; Warm ~ 29°C) for 6 weeks. Values are presented as mean ± SE for all fish from two replicate tanks per treatment. Significance established via analysis of variance and post-hoc analysis at $P < 0.05$. See text for values of initial and final mean weight.

The mean final weight of red drum utilized in the MMS assays of Experiment I were as follows: Cool, LE = 7.2 g; Cool, HE = 9.9 g; Ambient, LE = 8.6 g; Ambient, HE = 17.7 g; Warm, LE = 9.6 g; Warm, HE = 22.9 g. Marginal metabolic scope values ranged from 0.110 to 0.189 $L \cdot g^{-1} \cdot h^{-1}$. The pattern of MMS response to temperature was similar to that observed for growth, but there was no differential effect of feed energy (Figure 3.1). Analysis of variance confirmed no significant effect of feed energy on MMS (df = 11; $P = 0.988$). A significant difference in MMS among the levels of temperature, however, was resolved (df = 11; $P = 0.007$), with fish in the cool-temperature treatment (0.114 $L \cdot g^{-1} \cdot h^{-1}$) exhibiting lower MMS than those fish either in ambient- or warm-temperature treatments (0.151 and 0.178 $L \cdot g^{-1} \cdot h^{-1}$, respectively). Post-hoc testing revealed no significant difference in MMS between ambient- and warm-temperature treatments ($P = 0.074$). Also, there was no significant interaction between the effects of feed energy and temperature on MMS (df = 11; $P = 0.403$).

Because a significant effect of temperature on MMS was detected, further analysis of the components of MMS was performed. The median value of RMR for fish in the six treatments of this experiment ranged from 0.190 to 0.364 $mgO_2 \cdot g^{-1} \cdot h^{-1}$. As with MMS, feed energy did not significantly affect RMR (df = 11; $P = 0.147$). As well, no effect of the interaction between temperature and feed energy was detected (df = 11; $P = 0.510$). Temperature alone, however, did exhibit significant effects on RMR (df = 11; $P = 0.003$) of the red drum in this experiment. Post-hoc evaluation of the temperature effect revealed that RMR of fish in cool water (0.190 $mgO_2 \cdot g^{-1} \cdot h^{-1}$) was

significantly lower than RMR of fish in ambient-temperature water (0.313 $mgO_2 \cdot g^{-1} \cdot h^{-1}$; $P = 0.003$) and warm water (0.341 $mgO_2 \cdot g^{-1} \cdot h^{-1}$; $P = 0.001$).

The other component of MMS is LOCr, the oxygen concentration at which RMR becomes limited. Median LOCr values for fish in the different treatments ranged from 1.61 to 2.21 mg/L. No significant effect of temperature (df = 11; $P = 0.111$) or feed energy density (df = 11; $P = 0.129$) was detected in these data. As well, no significant interaction between feed energy and temperature (df = 11; $P = 0.105$) was resolved at the $P < 0.05$ level.

Modeling

Simulations of Experiment I using Ecophys.Fish resulted in an adequate match (i.e. coefficient of determination greater than 70 % between observed and simulated results) for growth rate, RMR, and MMS (Figure 3.2). The lowest MMSO value required to achieve adequate fit was obtained for fish in the cool treatment regardless of diet, 0.200; whereas, fish in the warm treatment consuming the LE diet required the largest value of MMSO, 0.260 (Table 3.2). Values of the required Winberg-adjustment ranged from 1.30 for fish in cool-water treatments to 1.50 for those in ambient- and warm-water treatments. Values of whole-body energy density at termination of simulations were 4.04 kJ/g for fish consuming the LE diet and 4.66 kJ/g for those consuming the HE diet.

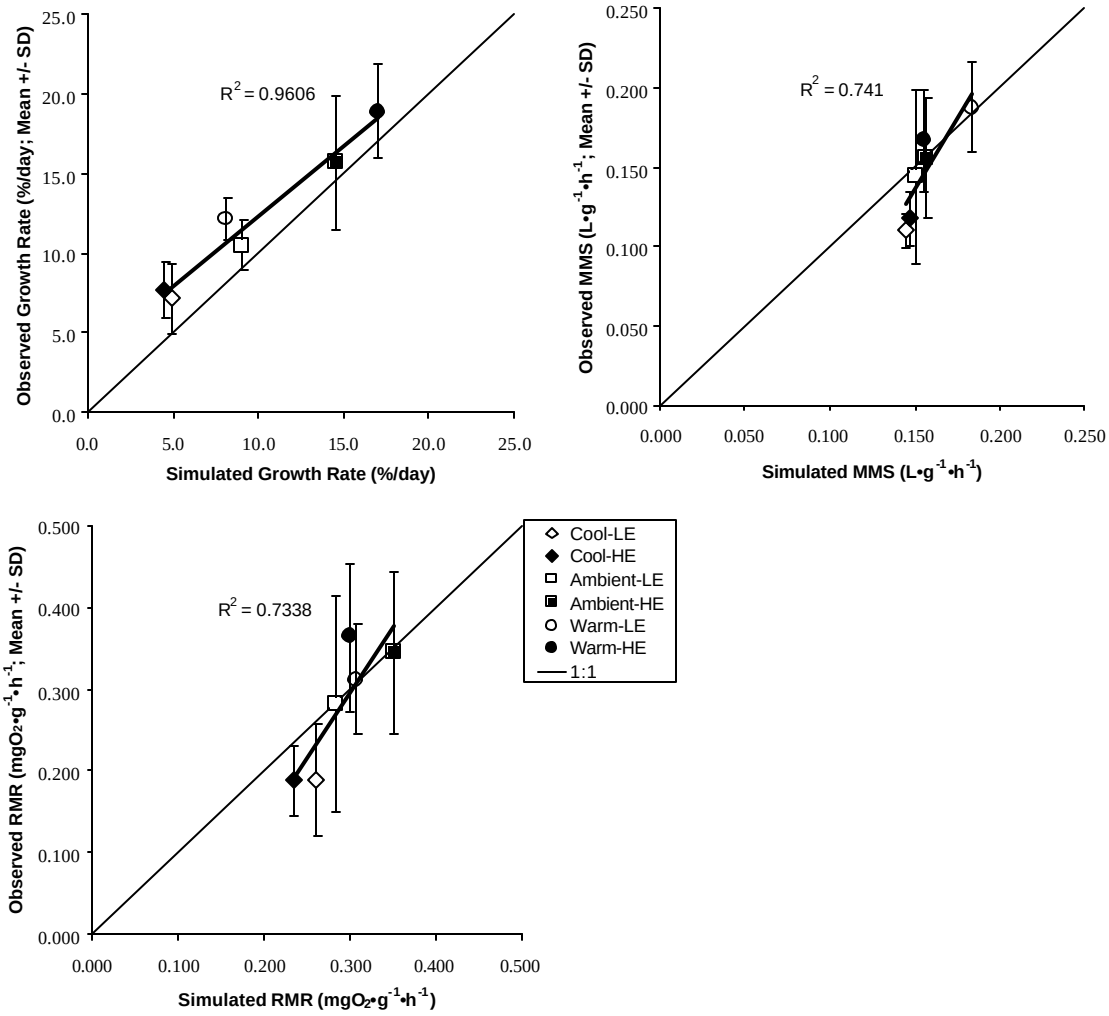

Figure 3.2. Comparison of observed versus Ecophys.Fish-simulated a.) growth rate (%/day; Mean +/- SD); b.) marginal metabolic scope (MMS; $L \cdot g^{-1} \cdot h^{-1}$; Mean +/- SD); and c.) routine metabolic rate (RMR; $mgO_2 \cdot g^{-1} \cdot h^{-1}$; Mean +/- SD) for red drum in Experiment I.

Table 3.2. Values of MMSO and Winberg-adjustment necessary to achieve adequate fit between observed and Ecophys.Fish-simulated fish performance for red drum in Experiment I.

Experimental Treatment		MMSO	Winberg-adjustment
Cool (~ 19°C)	LE	0.200	1.30
	HE	0.200	1.30
Ambient (~ 25°C)	LE	0.220	1.50
	HE	0.250	1.50
Warm (~ 28°C)	LE	0.260	1.50
	HE	0.250	1.50

Experiment II

Survival of fish fed the two diets exceeded 90 %, with no significant difference between treatments (Table 3.3). For those factors and performance measures common to the two experiments, results from this experiment were consistent with those observed in Experiment I: Growth rate was markedly greater for the HE diet than for the LE diet; however, metabolic capacity did not differ consistently between dietary energy levels. Juvenile red drum fed the LE diet had significantly lower feed efficiency (0.88 g gain/g fed) than those fed the HE diet (1.09 g gain/g fed; Table 3.3). Consistent with the results observed in Experiment I, growth rate was markedly greater for the HE diet (26.4 %/day) than for the LE diet (17.1 %/day). And, again, MMS did not differ between dietary energy levels; nor, in Experiment II, was there a between-diet difference in RMR or LOCr (Table 3.3).

Hepatosomatic index (HSI) and intraperitoneal fat (IPF) ratio for fish fed the LE diet were significantly lower than those of fish fed the HE diet; fish fed the LE diet had essentially no intraperitoneal fat (Table 3.4). Accordingly, whole-body lipid of fish fed the LE diet (2.2%) was significantly lower than that of fish fed the HE diet (3.4%); whereas, the moisture content of fish fed the LE diet was correspondingly greater than that of fish fed the HE diet (Table 3.4). Based on mean proximate composition, whole-body energy density was computed (Gatlin et al. 1986) as 3.88 and 4.72 kJ/g for LE and HE fish, respectively.

Table 3.3. Various performance measures for red drum fed the experimental diets in Experiment II [a].

Diet	Feed efficiency (g gain/g feed)	Survival (%)	Growth rate (%/day) [b]	Protein efficiency ratio (g gain/ g protein fed)	Protein conversion efficiency (g protein gain/g protein fed) × 100	Marginal metabolic scope (MMS; $L \cdot g^{-1} \cdot h^{-1}$)	Routine metabolic rate (RMR; $mgO_2 \cdot g^{-1} \cdot h^{-1}$)	Limiting oxygen concentration (LOCr; mg/L)
LE	0.88	90.00	17.08	1.72	28.7	0.170	0.395	2.32
HE	1.09 *	93.33	26.41 *	2.73 *	46.2 *	0.183	0.378	1.98
Analysis of variance, *Pr* >F	0.001	0.561	0.000	0.000	0.000	0.488	0.810	0.288
Pooled SE [c]	0.017	3.727	0.324	0563	0.034	0.006	0.014	0.086

[a] Values represent means of three replicate groups. Of the two values in each column, a larger value marked by "*" is significantly different from the other, at P = 0.05.
[b] Fish initially weighed 1.06 ± 0.15 g each.
[c] Pooled SE = vmean square error/number of replicates (Baker 1986).

Table 3.4. Body-condition indices and whole-body composition for juvenile red drum fed experimental diets in Experiment II [a].

Diet	HSI	IPF ratio	Whole-body composition (% of fresh weight)			
			Moisture	Protein	Lipid	Ash
LE	2.01	0.00	77.2 *	16.7	2.2	3.4
HE	5.41 *	1.86 *	73.1	16.9	3.4 *	3.9
Pr = F	0.000	0.000	0.001	0.513	0.012	0.164
Pooled SE[b]	0.226	0.150	0.366	0.357	0.193	0.232

[a] Values for HSI and IPF ratio represent means of 9 replicate fish from each treatment (n=9). Values of whole-body composition represent means of three replicate composite samples of three fish per aquarium (n=3). Of the two values in each column, a larger value marked by "*" is significantly different from the other, at P = 0.05.

[b] Pooled SE = v mean square error/number of replicates (Baker 1986).

Confinement-Stress Test

Fish had cortisol levels that varied dramatically among individuals, both within and between dietary treatments, such that any dietary effects could not be resolved (Figure 3.3). Significance values for pre-stress and post-stress were 0.369 (Student's *t* test), and 0.233 (nonparametric Mann-Whitney test), respectively. However, post-stress cortisol levels were higher than pre-stress levels ($P = 0.005$) for both feed treatments.

DISCUSSION

Results from Experiment I supported Ecophys.Fish predictions that red drum exposed to lower temperatures would exhibit lower growth rates and a general reduction in capacity for metabolic performance. Growth rates of those fish consuming the LE diet were lower than those of fish consuming the HE diet, except at the lowest temperature. Low temperature also reduced metabolic capacity as measured by MMS, but there was no significant effect of feed energy-density on metabolic capacity. Thus, feed energy did not limit metabolism in the same way that it limited growth.

Results supported the "energy/metabolism-tradeoff hypothesis." Temperature--but not dietary energy-density--had consistent and differential effects on metabolic capacity, as reflected in MMS. Higher temperatures increased MMS of the fish, thereby providing them more capacity to fully exploit the greater energetic and nutritional value of the HE diet for growth. But, the increase in RMR with increasing temperature also is consistent with the energy-bonus hypothesis (McLaren 1963; Brett 1971). The consistent lack of significant differences in growth and metabolic responses between the ambient- and warm-temperature treatments was not surprising given that juveniles

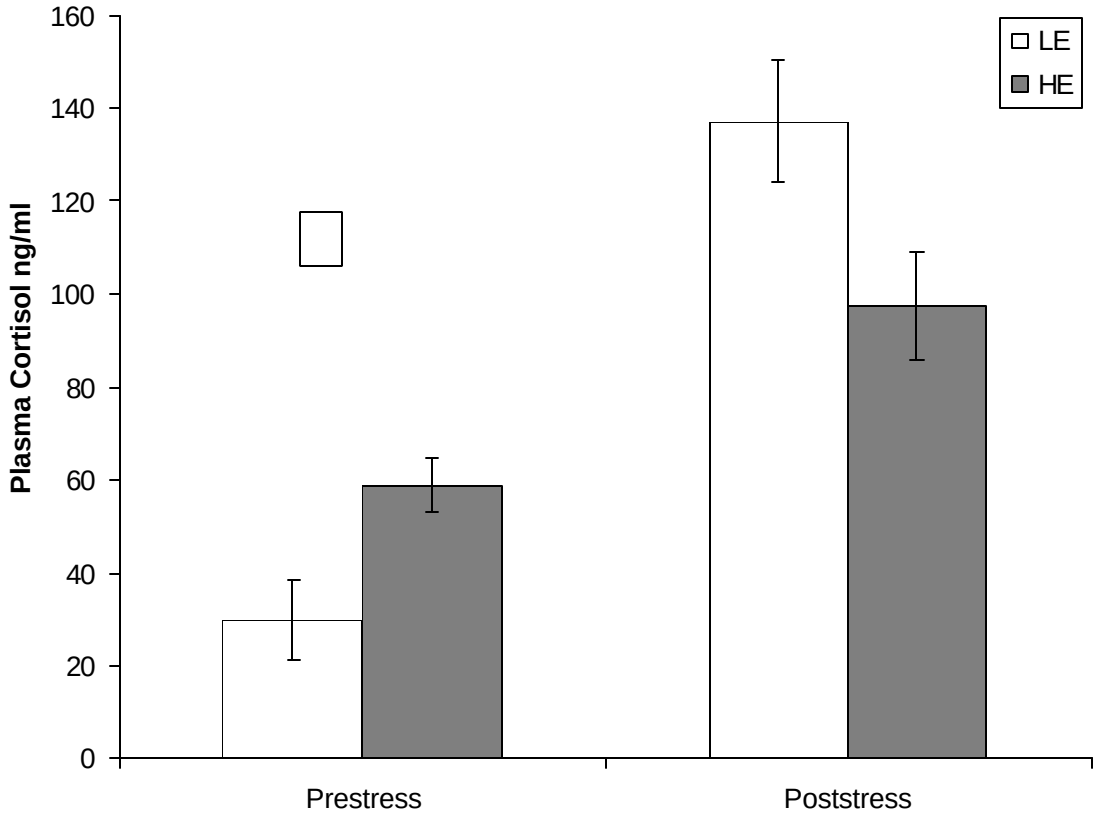

Figure 3.3. Serum-cortisol concentrations in red drum before confinement stress and 15 min after confinement stress. Concentrations are presented as mean ± SE for samples of 9 individual fish per treatment.

of this species inhabit highly-variable coastal ecosystems (Peters 1987; Matlock 1990). Increasing the warm temperature treatment to 31°C in order to maintain a uniform ~ 6 °C difference among cool, ambient, and warm treatments would have been methodologically-appropriate and, presumably, may have induced a significant decrease in growth; however, our intention was to evaluate the energy/metabolism-tradeoff hypothesis and not to assess effects of exposing red drum to temperatures approaching their upper-lethal tolerance limits (Neill 1990; Procarione and King 1993).

Because effects in Experiment I were potentially confounded in that those treatments which supported greater metabolic capacity also produced larger fish—whose metabolic rates tend to be declining functions of body weight (Fry 1947; Brett and Groves 1979; Neill et al. 2004)—Ecophys.Fish was employed to help interpret the results. The parameter MMSO is used in Ecophys.Fish to quantify the inherent metabolic efficiency of the fish-environment system once the interacting effects of temperature, DO, salinity, pH, feed energy-density, and feed digestibility have been accommodated (Neill and Bryan 1991; Neill et al. 2004); whereas, the Winberg-adjustment provides a means with which to adjust the fraction of metabolic scope used for routine metabolism beyond that used for standard metabolism (Winberg 1960; Neill et al. 2004). Because utilizing Ecophys.Fish in this way does not represent true "experimentation," it is not appropriate to statistically evaluate variation in either MMSO or the Winberg-adjustment; instead, a more straightforward comparison of observed versus simulated results and an assessment of the parameters necessary to achieve adequate fit was used to elucidate relationships within the data.

Operating within the assumptions of Ecophys.Fish, and using as model inputs the observed environmental and nutritive regimes, we were able to achieve a high degree of agreement (> 73 %) between observed and simulated fish performance in Experiment I. As reflected in the empirically-obtained significant effects of feed energy and temperature—and their interaction—on growth rate, the model appears to have captured and further resolved the metabolic-advantages imparted to the fish, as well. Similarly, terminal values of whole-body energy density obtained from simulations of Experiment I were congruent with values estimated from the proximate composition of fish from Experiment II. The implications of the modeling results are that those fish in warmer water will inherently have a larger capacity for performance (as measured by larger values of MMSO and the Winberg-adjustment), but this capacity may not be realized unless they are consuming a diet with sufficient energy availability.

Growth and metabolic capacity of the fish in Experiment II followed the pattern established in Experiment I. Differences in experiment duration, feeding frequency, and group size made direct comparison of quantitative results between experiments inappropriate. However, Experiment II provided important corroborating evidence that energy-density of the LE diets, per se, was what limited growth of juvenile red drum at ambient temperature. Recall that all fish in this experiment were fed to apparent satiation twice daily, using a feeding table with application rates based on dry weights of ration; this assured that as-fed weights of the LE diet were almost four times the as-fed weights of the HE diet offered to the equivalent biomass of fish. Yet, the hepatosomatic index (HSI), intraperitoneal fat (IPF) ratio, and whole-body lipid content of red drum fed

the LE diet were markedly lower than those of fish fed the HE diet. Red drum fed the LE diet had no evident IPF, which is very uncommon for aquacultured red drum (Serrano et al. 1992; Moon and Gatlin 1994; Webb and Gatlin 2003; Li et al. 2004), and even for wild populations (Craig et al. 2000). These observations confirmed the insufficiency of utilizable energy from the LE diet.

So, how are wild red drum able to grow rapidly and fatten on a diet of natural forage, which our LE feed was designed to emulate? In fact, red drum are not able to grow as rapidly on natural forage as on highly digestible, high-energy prepared diets (Wurts and Stickney 1989; Serrano et al. 1992; Lee 1997). But, another component of the answer is that natural forage tends to be more highly digestible than even our LE and HE diets (which had approximately equal energy digestibilities), and probably much more digestible than commercial pelleted feeds (Grey, 2003). Under Ecophys.Fish, high digestibility of diet can compensate for reduced energy density.

Agar occasionally has been used to bind markers to natural foods for digestibility studies with fish (Wetherbee and Gruber 1993) and was used in this study to manage the LE diet for low energy-density and high moisture. We did not notice any depression of apparent appetite or feed intake for fish fed the LE diet, relative to those fed the HE diet. Daily intake of 6-7 % of body weight on a dry-weight basis was equal to or exceeded the published rates for juvenile red drum of comparable size fed dry diets (Webb and Gatlin 2003; Li et al. 2004).

The effects of nutrition on circulating cortisol concentration are generally without evident patterns thus far, although low feed intake leading to protein and energy

malnutrition has been proven to increase cortisol release in mammals (Soliman et al. 2000; Kilic et al. 2004). In this study, however, there was not a significant difference in cortisol titers between fish fed the LE and HE diets. Periods of chronic stress leading to reduced growth do not always coincide with higher levels of circulating cortisol; instead, cortisol, as a primary stress response, tends to better serve as an indicator of acute environmental stressors (Van Weerd and Komen 1998; Pérez-Domínguez and Holt 2006). As such, the 15-min confinement stress test induced a significant increase in cortisol level. Metabolic capacity appears to be more sensitive to recent life history events than to the chronic stress of malnutrition (Fry 1971; Barton and Iwama 1991; Tomasso 1996). Metabolic capacity as a function of confinement stress—or as a correlate of cortisol level—was not measured, but no effect of dietary energy on metabolism was resolved. Like the other metrics of bioenergetic well-being, however, growth rate was markedly affected by dietary energy-density.

CHAPTER IV

EFFECTS OF DISSOLVED-OXYGEN, TEMPERATURE, AND FEED ENERGY ON PERFORMANCE OF JUVENILE RED DRUM

SYNOPSIS

Understanding how interactions of environment and feed energy affect fish performance is important for optimizing aquacultural production as well as for estimating the impacts of environmental challenges encountered by wild fish. In well-oxygenated waters, a dependency of growth and metabolism on environment and nutrition has been demonstrated for juvenile red drum, with feed energy limitations developing at higher temperatures and metabolic-capacity limitations developing at lower temperatures. Here, I report on experiments that incorporated manipulation of dissolved-oxygen concentration (DO) into four environmental regimes designed to evaluate growth and marginal metabolic scope (MMS) of juvenile red drum consuming feeds with two levels of gross dietary energy—low energy (LE) and high energy (HE), with ~ 4.1 and ~ 15.9 kJ/g as-fed, respectively: 1) two levels of temperature (18.5 C and 28.5 C) with static DO near air-saturation; 2) two levels of DO (low DO = 25-40 % of air-saturation, and high DO = near 100 % air-saturation) with constant low (~ 18.5 C) temperature; 3) two levels of DO (low DO and high DO) and constant high (~ 29 C) temperature; and, 4) temperature (~20 to 28 C) and DO (low DO to high DO) cycling in phase, with a 24-h period. After 4 weeks imposition of the first treatment regime, results conformed with expectations that growth rate and MMS would be depressed at the lower temperature and that growth rate at the higher temperature would be greater for fish

consuming the HE diet. At the lower temperature, limiting effects of feed energy remained evident in the metabolic response. At the higher temperature, low DO was limiting, and the limiting effects of low feed energy on performance were amplified. Additionally, at the higher temperature, an energetically deficient diet appeared to buffer against the effects of low oxygen on red drum and to provide them with metabolic capacity that would have been ample under improved environmental conditions. Within the cyclical environment, growth rate remained an energy-dependent response; however, the specific cyclic diel environment did not promote anticipated enhancement of growth. Subsequent to laboratory experiments, the simulation model Ecophys.Fish was employed to further explore and interpret observed outcomes. Findings of this study provide insights as to how the euryhaline red drum is so well-adapted to its naturally variable environment.

INTRODUCTION

Hatchery-based stock enhancement is gaining respect as an integral component of fisheries conservation efforts. Effective stock enhancement programs typically incorporate a multi-faceted approach that involves not only the release of hatchery-produced fish but also fishery monitoring, policy optimization, education, and outreach, in order to achieve conservation and sustainability of the target species and its ecosystem (McEachron et al. 1993; Vega 2003; Jenkins et al. 2004). While specific strategies and tactics are debatable, there is general agreement that successful stock enhancement efforts are adaptable, holistic, and self-critical; they carefully consider the numerous species-, ecosystem-, and culture-specific factors unique to the enhancement of a

particular species in a given habitat, and respond accordingly (Munro and Bell 1997; Masuda and Tsukamoto 1998; Blaxter 2000; Fushimi 2001; Mustafa 2003; Mustafa et al. 2003).

Among the factors fundamental to ensuring the proper development of hatchery-produced fish are appropriate rearing and production techniques, which include providing nutritionally adequate forage or prepared feeds, and a favorable physiochemical environment (Vega 2003). Hatchery facilities incorporate highly sophisticated new technologies and traditional aquacultural practices as a means of blending natural and controlled environmental conditions in order to optimize health and performance of fish prior to their release into the wild. Extensive research is performed to scrutinize on-going efforts and refine the procedures and facilities used in fish production. One of the greatest challenges associated with the production of red drum and other estuarine species is that seasonal variation in water temperature—in particular, low temperature—increases mortality and decreases growth (Thacker and Griffin 1994; Scharf 2000). It has been suggested, however, that depressed red drum performance during the colder months is associated with the lack of food availability during this season and not a direct effect of the lower ambient temperature (Hopkins et al. 1988). Regardless of the underlying mechanism, undersized fish have been found to exhibit relatively low rates of survival, both in the wild and in hatchery settings (Stunz et al. 2002; Vega 2003; Lorenzen 2006). Such findings emphasize the need to better understand red drum growth—and to determine optimal growing conditions—under various environmental and foraging regimes.

Physiologically, various factors interact to influence the growth and overall health of fish. These factors include but are not limited to environmental conditions, a proper balance both of nutritive and non-nutritive dietary components that enhance immunity and disease resistance, and feeding practices (Gatlin 2002a; Fontaine et al. 2007). In particular, fish performance reflects a dynamic balance between supplies of oxygen and energy-yielding substrates for metabolism (Fry 1947; Brett 1979; Neill et al. 2004). The balance has been set by evolution to give best performance under an individual's typical environmental and physiological circumstances. Increased feed energy tends to shift performance optima towards higher temperatures, where more metabolic scope is available (Fry 1947; McLaren 1963; Brett 1971; Brett 1979; Azevedo et al. 1998; Gillooly et al. 2001; Neill et al. 2004; Fontaine et al. 2007). Some studies have suggested that optimally cycling temperature and DO regimes may further increase metabolic scope and consequent growth in certain fish species (Hubbs 1964; Hokanson et al. 1977; Dickerson and Vinyard 1999). Early work with Ecophys.Fish—a computer-based simulation model of fish performance in time-varying environmental regimes—indicated that for red drum any extra metabolic capacity is useful only if available feed energy also is increased, perhaps to levels above approximately 10.5 kJ/g (Neill et al. 2004). While few natural forages available to red drum have this much energy, many prepared feeds have over 16 kJ/g and some even more than 20 kJ/g. Simulations with Ecophys.Fish further suggested that the proper cyclical environment might result in a decrease in the acclimation state of DO, thereby increasing active metabolic rate, and,

consequently, metabolic capacity during the high-temperature, high-DO phase of the cycle (Neill et al. 2004).

Preliminary experiments in this series have provided some evidence in support of these hypotheses (Neill et al. 2004; Fontaine et al. 2007); however, these experiments were limited to manipulations of temperature and feed energy only. In an effort to better resolve the effects of environment and feed energy on juvenile red drum performance, I incorporated contrasts in DO—in addition to those in temperature and feed energy—into several laboratory experiments, with four distinct regimes of environment. Specifically, I examined growth and metabolic performance under conditions of 1) constant high DO with varying temperature and feed energy, 2) constant low temperature with varying DO and feed energy, 3) constant high temperature with varying DO and feed energy, and 4) cycling temperature and DO with varying feed energy.

Subsequent to the laboratory experiments, Ecophys.Fish was employed to further elucidate observed experimental results. Complementation of traditional statistical analyses with simulation modeling (Clark 2003; Vega 2003; Neill et al. 2004; Fontaine et al. 2007) provides a means by which to explore complex ecophysiological interactions and relationships that may otherwise elude interpretation, especially under transient-state conditions.

METHODS

Preparation of Experimental Diets

The two experimental diets were prepared and stored following Fontaine et al. (2007). Briefly, the high-energy diet (HE) is a highly nutritious, dry pelleted-feed originally developed for research purposes and designed to contain 10% moisture, 40% protein, 10% lipid and an estimated 15.9 kJ digestible energy (DE) per gram on a dry-weight basis. This formulation meets or exceeds all known nutritional requirements of red drum and most warmwater fishes (NRC 1993; Gatlin 2002b; Webb and Gatlin 2003; Li et al. 2005). The low-energy diet (LE) was designed to contrast with the HE diet and to more closely resemble the red drum's natural forage in terms of moisture, protein, lipid, and energy content. This low-energy alternative was formulated to contain 80% moisture, 12% protein, 2% lipid and an estimated 3.3 kJ DE/g on a fresh-weight basis.

Static Environment - Experiment I: High Dissolved Oxygen at Low and High Temperature

In September 2004, juvenile red drum (*Sciaenops ocellatus*) were obtained from Texas Parks and Wildlife Department's Marine Development Center (TPWD – MDC) in Flour Bluff, TX, and were subjected to a 2-week conditioning period in a brackish-water, recirculating system at Texas A&M University's Aquacultural Research and Teaching Facility (ARTF). A commercial maintenance diet was fed to all the fish during the conditioning period, after which individual fish averaging approximately 1 g were graded by size and stocked into cages, one individual per cage, within larger tanks. From this time onward, the fish in each cage was fed only one of the two experimental

diets. Within the following week, any dead fish was replaced; and, at week's end, the initial weight of each individual was determined—1.5 g ± 0.51 (mean initial weight of all individuals from experiment I ± SD).

Except in cycle experiment II (see below), cages of the design described by Fontaine et al. (2007) were employed throughout this study. Cages were distributed in circular, 1136-L tanks, each with independent, self-contained biofiltration. Each cage consisted of a submerged 19-L polyethylene bucket and lid; both were modified with 0.5-cm plastic mesh, covering ~16-cm diameter holes. Resting near the bottom of every cage an air stone encouraged upwelling of water from the bottom to the top of the cage. Cages were secured to a "false-bottom" made from egg-crate louver and supported by plastic blocks ~6 cm above the tank bottom. Six replicate cages of fish per diet were exposed to each environmental treatment regime. Growth was monitored weekly by removing and weighing the individual fish. Growth performance was evaluated by calculating percent change in weight per day (%/day), using the difference between final and initial fish weight, divided by initial weight and the number of days the fish spent in the cage.

Water flow-rate through each biofilter was maintained at approximately 1,120 L/h via an aquarium powerhead that maintained adequate water quality (total ammonia nitrogen = 0.4 mg/L) through biological filtration. Twice daily, fish were fed in excess via a "feeding tube" that protruded from above the surface of the water to ~ 8 cm into each cage. The simulation model Ecophys.Fish was utilized in conjunction with observation to ensure that fish were presented with excess quantities of diet (Neill et al.

2004; Fontaine et al. 2007). Uneaten diet and expelled feces were removed from each experimental tank on a weekly basis. Cages were similarly cleaned at each weighing event. Salinity was maintained at 10.9 ppt ± 0.5 by adding synthetic sea salt (Fritz Industries Inc., Dallas, TX) to freshwater from an on-site well. After each weighing event, ~30 % of the water in each experimental tank was flushed and replaced to prevent water-fouling that might otherwise have resulted from the high feeding rate. A 12 h light:12 h dark photoperiod was maintained with fluorescent lights controlled by timers. The low-temperature treatments were controlled by electric chillers and the high-temperature treatments were achieved via multiple submerged electric aquarium heaters controlled by mercury-contact thermometers (Table 4.1).

Following Springer and Neill (1988), Neill and Bryan (1991), Fontaine (2002), Clark (2003), and Fontaine et al. (2007), automated routine respirometry was used to measure metabolic performance of red drum after exposure to the experimental treatments. Thirty-two days after the start of the experiment, I obtained for each fish a median value of routine metabolic rate (RMR) adjusted for biological and chemical oxygen demand (BCOD, $mgO_2 \cdot g^{-1} \cdot h^{-1}$); limiting oxygen concentration (LOC, mg/L) for the observed RMR, and their ratio, marginal metabolic scope (MMS, $L \cdot g^{-1} \cdot h^{-1}$). MMS was calculated for each fish, as the median value for RMR and LOC pairs (Neill and Bryan 1991; Springer and Neill 1988; Fontaine et al. 2007). Fish fasted for 24 h prior to their placement into a respirometer. Respirometry was conducted within the experimental tanks where the fish had been caged. Low-level fluorescent lighting placed above the respirometers mediated potential effects of

Table 4.1. Treatment designation and values for the various regimes of temperature (Low Temp, High Temp, Cyclical) and dissolved oxygen (Low DO, High DO, Cyclical) as presented to juvenile red drum during each experiment.

Environmental Regime		Temperature (mean C ± SD)	Dissolved Oxygen (% saturation; mean mg/L ± SD)
Static Environment Experiment I			
High DO -	Low Temp	18.6 ± 0.6	~88%; 7.5 ± 0.3
	High Temp	28.2 ± 0.7	~74%; 5.4 ± 0.5
Static Environment Experiment II			
Low Temp -	Low DO	18.6 ± 1.1	~27 %; 2.3 ± 0.5
	High DO	18.2 ± 0.9	~85 %; 7.2 ± 0.3
Static Environment Experiment III			
High Temp -	Low DO	29.1 ± 0.6	~36%; 2.6 ± 0.6
	High DO	29.1 ± 0.6	~78%; 5.5± 0.4
Cyclical Environment Experiment I		20.5 – 27.5 (range)	~25 – 75%; 2.0 – 5.5 (range)
Cyclical Environment Experiment II		20.0 – 28.0 (range)	~25 – 76%; 2.0 – 5.5 (range)

photoperiod on metabolic rate while not disturbing the remaining fish. Final fish weight was obtained immediately prior to placement of fish within the respirometer chamber. After ~ 20 h of respirometric measurements, fish were removed from the chambers, euthanized, and preserved in cold storage (-20 C). Immediately following each "fish run", the BCOD was estimated with a 1 hour "blank run" in the corresponding empty respirometer chamber.

Statistical Analyses

Differences in treatment means were evaluated using SPSS and considered significant at $P < 0.05$ for all analyses. Growth and metabolic results were based on the response of an individual fish in each of six replicate cages per diet-environment treatment combination. While the assumption of equal error variances was violated for growth rate, the analysis of variance tests utilized were sufficiently robust that such violation was unlikely to threaten the validity of the results, following the findings of Milliken and Johnson (1992). Additionally, the more critical assumptions of normally distributed error variance with a mean of zero were not violated; thus, the results of the analysis remained informative and convincing.

Static Environment - Experiment II: Low Temperature with Low and High Levels of Dissolved Oxygen

In May 2005, I examined the response of red drum in an environment of constant temperature but differing levels of DO (Table 4.1). Due to concerns about potential adverse effects of isolation on fish behavior (Kendal et al. 2004), multiple fish were stocked into each cage in Experiment II versus the single fish per cage in Experiment I.

Otherwise, the experimental design, observed values of environment, and monitoring of performance were nearly identical to that of Experiment I. Here as well, fish were obtained from the TPWD – MDC in Flour Bluff, Texas. Following a 2-week conditioning period, four fish were stocked into each cage; initial weight of individual fish was 2.0 g ± 0.3 (mean ± SD); individual fish weights were obtained weekly thereafter. At the bottom of each cage was a refuge (Figure 4.1) constructed of short segments of polyvinylchloride (PVC) pipe, 25.4 mm (inner diameter). Refugia were intended to shelter smaller individuals from aggression by their larger cage-mates, by providing shaded areas and overhangs.

Maintaining DO at non-air-saturation values for an extended period of time required control measures beyond simply valving the flow of air from low-pressure electrical blowers. Target oxygen levels for low-DO treatments were ~ 25 % of air saturation and, for high-DO treatments, ~ 80 % air saturation. Low-DO treatments were achieved by "stripping" the oxygen from the water column with nitrogen gas. Effervescing N_2 from a 160-L liquid-nitrogen tank was mixed with fresh air from the blowers until the desired gas-mix was achieved. Twice-daily adjustment was sufficient for maintenance of DO within ~ 1.5 ppm of target values. As before, the temperature in the low-temperature treatment was maintained at ~ 18.5 C via electric chillers.

Submerged powerheads were used to ensure even mixing of oxygen-enriched or oxygen-depleted, temperature-treated water within the experimental aquaria. Salinity was maintained at 14.0 ppt ± 0.41 with the addition of synthetic sea-salts and well-water. Six replicate cages of fish fed each of the two diets were exposed to each environmental

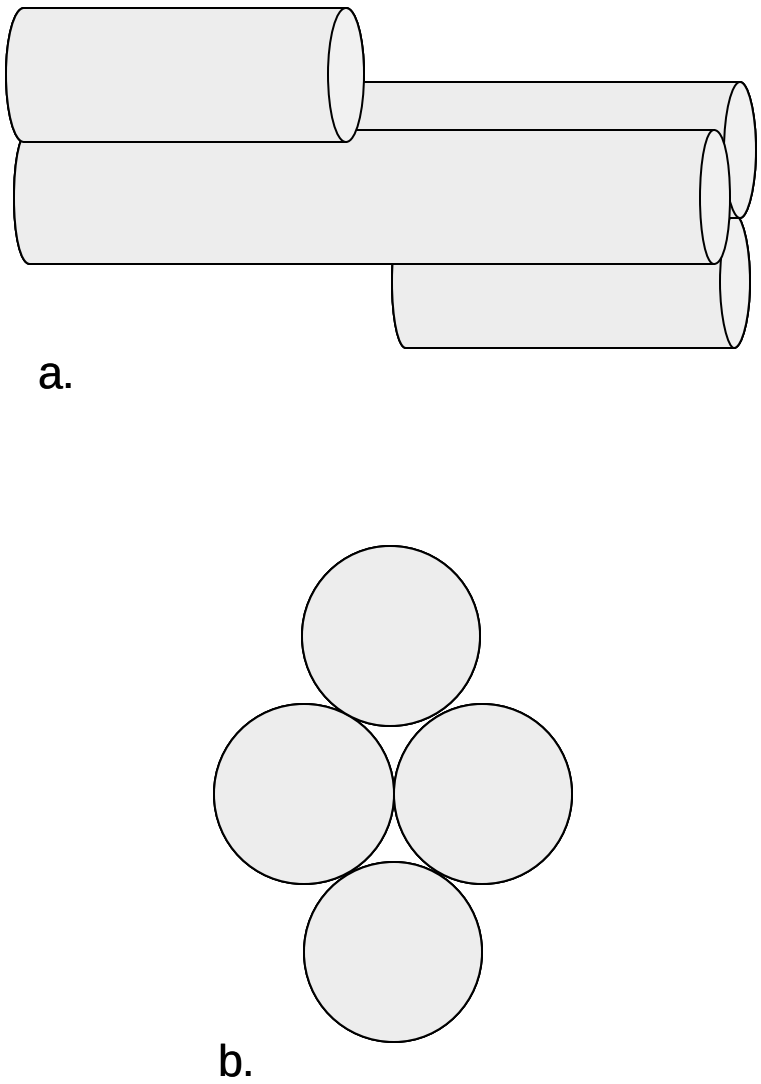

Figure 4.1. Side (a) and cross-sectional (b) views of the refuge used to provide smaller individual red drum with shelter from larger individuals. Refugia were constructed of four pieces of 25.4 mm (inner diameter) PVC pipe glued together as shown. Short pipes were 76.2 mm in length, and long pipes were 152.4 mm in length. Drawing not to scale.

treatment. After 25 days, respirometry assays were initiated as previously described for Experiment I. Respirometry was conducted in a neighboring tank held at the same low temperature (~18.5 C); this tank shared the primary source of water used in all treatments. Oxygen levels in the water bath used for respirometry were maintained near air saturation.

Statistical Analyses

Analyses were based on the response of the median fish in each cage, with six replicate cages per diet-environment combination, and using SPSS with significance at $P < 0.05$.

Static Environment - Experiment III: High Temperature with Low and High Levels of Dissolved Oxygen

In an effort to provide a contrasting temperature regime with which to examine effects of varying DO on red drum performance, a final constant-temperature experiment was conducted with larger groups of fish and under the conditions of a more conventional laboratory feeding trial. Specifically, effects of feed energy and DO were assessed under warm-water conditions. This final experiment was performed in July 2005 with juvenile red drum obtained from the TPWD – Sea Center Texas facility in Lake Jackson, Texas.

Twenty fish with an average weight of 1.0 g were stocked into 110-L glass aquaria in a closed, recirculating system and subjected to a 2-week conditioning period during which they were fed a commercial maintenance diet. Experimental diets and DO treatments were randomly and evenly assigned across aquaria but temperature was held

constant at ~ 29 C, which has produced high rates of growth in previous studies (Neill et al. 2004; Fontaine et al. 2007). As with Experiment II, the low-DO treatments were achieved by bubbling nitrogen gas (from liquid-nitrogen tanks) though air-saturated water returning from the biofilter; in the high-DO treatments, air from low-pressure blowers was used in lieu of nitrogen (Table 4.1). Here as well, target oxygen levels for low-DO treatments were ~ 25 % of air saturation and, for high-DO treatments, ~ 80 % air saturation. Salinity was maintained at 14.4 ppt ± 0.9 as previously described.

Fish were fed in excess using both Ecophys.Fish and observation to ensure that the amount of feed presented remained adequately high without presenting an excessive load to the biofilter. Environmental parameters were monitored at least twice daily and adjustments to gas- and temperature-control apparatus were made as necessary. Fish from each aquarium were weighed as a group on a weekly basis; uneaten feed and waste were siphoned at this time. Respirometric assays were initiated 28 days from the start of the trial and were conducted in vacant aquaria within the culture-system; DO in the aquaria used for respirometry was maintained near air saturation. Individual fish from each treatment were randomly chosen, isolated, and required to fast for 24 h prior to placement into the respirometer. Final weights were obtained immediately prior to fish placement within the respirometer chamber.

Statistical Analyses

Analysis of variance tests with SPSS were based on the response of the median fish from each replicate aquarium (n = 16) and deemed significant at $P < 0.05$. As in Experiment I, the assumption of equal error variances was violated for growth rate;

however, since no other assumptions were violated, the results are considered legitimate and informative (Milliken and Johnson 1992).

Cyclical Environment: Diel Cycling Temperature and Dissolved Oxygen

I sought to evaluate the effects of cyclical regimes of temperature and DO on juvenile red drum with two experiments that ran concurrently with the constant-environment experiments I and II described above. Fish from cycle experiment I were from the same batch as those from constant experiment I. Those from cycle experiment II were from the same batch as those from constant experiment II. The aquaria used for cyclical experiment I consisted of 1136-L culture systems and cages with the self-contained bio-filtration units described above. Following the procedures used in the constant-environment Experiment I, one fish per cage (1.6 g ± 0.32; mean wt ± SD) was stocked in Cycle Experiment I. Cycle Experiment II differed slightly in that two replicate 1136-L culture systems were subdivided into two compartments each via egg-crate louver covered in 0.5-cm plastic mesh. Each of the four compartments was outfitted with pumps to encourage water exchange between the sides but to prevent mixing or escape of fish. Fifty individuals with an average weight of 1.0 g (determined via group weighing) were stocked into each replicate compartment.

Temperature and DO treatments were presented as in-phase diel cycles, each with a 24-h period and amplitures intended to approximate the range achieved in the static-environment experiments (Table 4.1). The cyclical regimes of temperature and DO were achieved via the methodology described in Chapter II. Briefly, an environmental monitoring probe was used to gather data within the experimental

aquaria, and a microcomputer interpreted those data. To achieve target values of temperature and DO that were proscribed by the user, pumps and solenoid valves controlled temperature and DO, respectively. Due to occurrence of low-DO conditions inherent under the cyclical regime, respirometry on the fish from the cyclical experiments was conducted in a neighboring tank which shared the primary water source used throughout the experiments. Here, temperature was held constant at a level between the extremes achieved in the experimental aquaria and DO was maintained near air saturation.

Dietary treatments in both cycle experiments were similar to those employed in the constant experiments. Fish were fed—in excess—the same LE and HE diets as described above; in each cycle experiment, half of the cages/compartments were randomly assigned one of the two dietary-energy treatments.

Statistical Analyses

In each of the cyclical-environment experiments, responses were pooled within feed energy treatment. In cycle experiment I, analysis of variance was used to evaluate per-cage responses; whereas, in cycle experiment II, responses from all individuals in a replicate compartment were pooled within treatment and subjected to Mann-Whitney analysis because of non-normal error variances. Analyses were performed with SPSS, and differences in treatment means were considered significant at $P < 0.05$.

Modeling

Growth and metabolic performance of red drum in all experiments was simulated using Ecophys.Fish (Neill et al. 2004). For each simulation, the mean environment (temperature, DO, salinity, pH) from replicate tanks each hour was interpolated from observed data as recorded from all experimental tanks within the same environmental treatment. In the case of the static-environment Experiment III and cyclical-environment Experiment II—both of which involved multiple aquaria/tanks with replicate temperature treatments—the model's environmental inputs were based on the mean of the replicate treatments. As well, comparisons of growth and metabolic data as output from the model were based on the same subsets of data and have the same dimensions of measurement as the statistical analysis from the corresponding experiment.

Modeling assumptions and procedures were based on those initially developed by Neill et al. (2004) and subsequently refined by Fontaine et al. (2007) in that manipulation only of MMSO and the Winberg-adjustment parameters was allowed. Iterative simulation of each experiment progressed until an optimum match between observed and simulated pairs of RMR, MMS, and growth rate were achieved (Fontaine et al. 2007). In this study, a match was considered optimum only when simulated values of RMR, MMS, and growth rate were simultaneously within a 95 % confidence interval on observed values. Assumed energy densities of fish (GEFish) and feed (GEFeed) followed Fontaine et al. (2007).

RESULTS

Static Environment - Experiment I: High Dissolved Oxygen at Low and High Temperature

In the "constant" high-DO environment, growth rate increased with temperature and feed energy, but interaction of temperature and feed energy did not have a significant effect on growth (Table 4.2). Growth rate varied from 2.29 to 16.98 %/day across all high-DO treatments, with the growth rate of juvenile red drum at low temperature (3.45 %/day) significantly lower than that of their counterparts at high temperature (10.65 %/day).

Marginal metabolic scope (MMS) was used as the primary measure of metabolic capacity for performance. MMS is defined as the ratio of the routine metabolic rate (RMR) to the limiting oxygen concentration for that rate (LOCr). In general, a larger MMS value suggests a greater capacity for physiological performance under conditions of the test environment. As with growth, a significant effect of temperature was detected; contrary to observed growth relationships, there were no significant effects of feed energy or its interaction with temperature (Figure 4.2). Marginal metabolic scope ranged from 0.108 to 0.304 $L \cdot g^{-1} \cdot h^{-1}$, with the mean MMS of fish in cool water (0.140 $L \cdot g^{-1} \cdot h^{-1}$) significantly lower than mean MMS of fish in warm water (0.240 $L \cdot g^{-1} \cdot h^{-1}$).

Because the effect of temperature on MMS was significant, further analysis of the components of MMS was performed. The median value of RMR for fish in a high-DO environment and subjected to the six treatments of this experiment ranged from 0.127 to 0.536 $mgO_2 \cdot g^{-1} \cdot h^{-1}$. As with MMS, temperature was the only significant factor

Table 4.2. Performance of juvenile red drum exposed to constant high dissolved oxygen with two levels of temperature, and with two levels of feed energy-density, during a 4-wk feeding trial (Experiment I).

Environmental Regime		Diet	Growth rate (%/day [a])	Marginal metabolic scope ($L \cdot g^{-1} \cdot h^{-1}$)	Routine metabolic rate ($mgO_2 \cdot g^{-1} \cdot h^{-1}$)	Limiting oxygen concentration (mg/L)
High DO	Low Temp	LE	2.93	0.132	0.279	1.98
		HE	3.98	0.148	0.228	1.54
	High Temp	LE	9.13	0.219	0.397	1.81
		HE	12.18	0.262	0.498	1.92
Analysis of variance, *Pr* > *F* [b] :						
Temperature			0.000 *	0.000 *	0.008 *	0.706
Feed energy			0.015 *	0.071	0.661	0.582
Temperature x Feed energy			0.204	0.376	0.203	0.367
Standard Error			0.718	0.001	0.002	0.064

[a] Fish initially weighed 1.5 g ± 0.51 (mean ± SD).
[b] Significance [*] probability associated with the *F*-statistic for an analysis of variance of the stated factor; *Pr* > *F*.

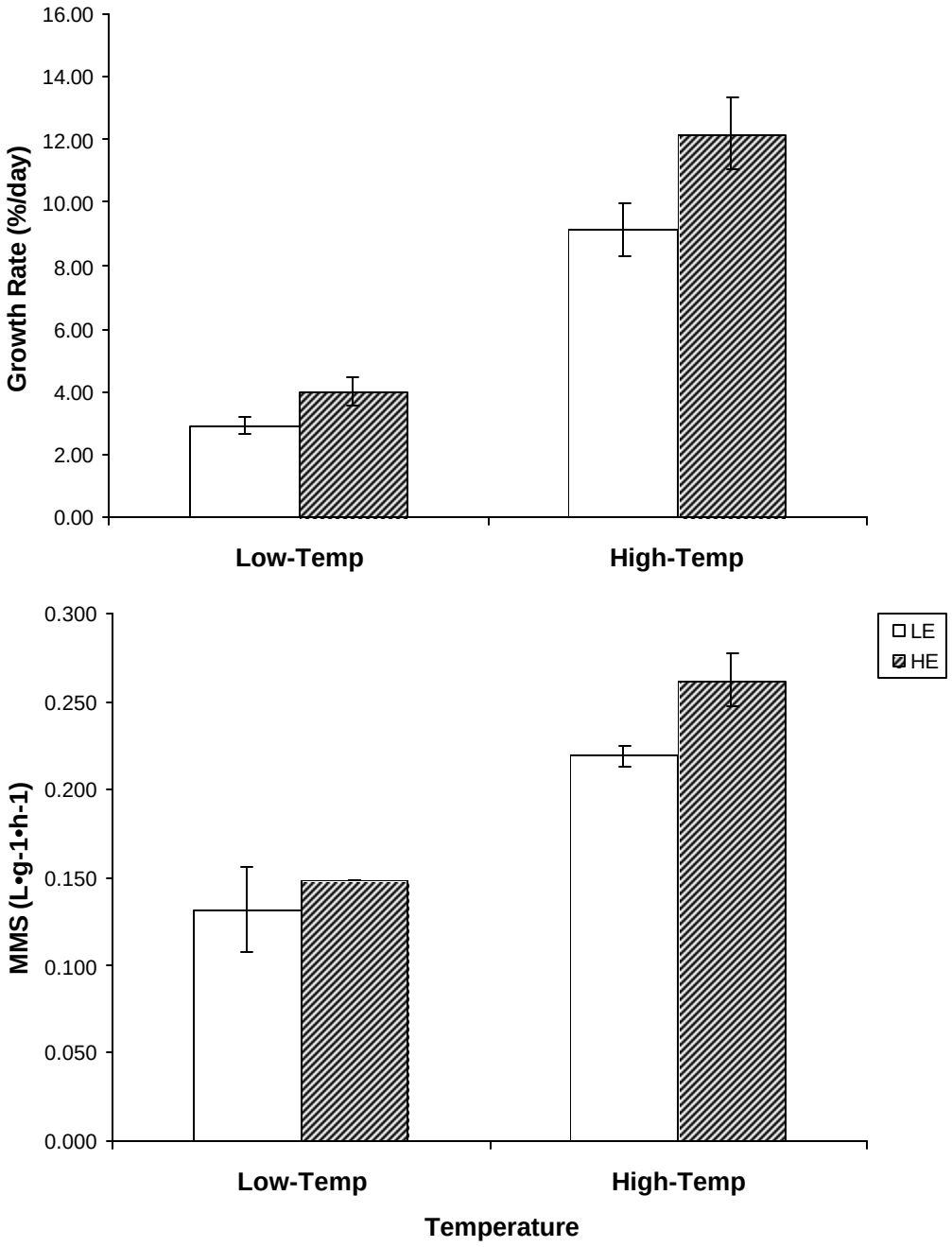

Figure 4.2. Growth rate (%/day) and marginal metabolic scope (MMS; $L \cdot g^{-1} \cdot h^{-1}$) for red drum in cool water (~ 19 C) and warm water (~28 C) while consuming a low-energy diet (LE) or a high-energy diet (HE) under high-DO conditions for a period of 4-wk during Experiment I. Values are presented as mean ± SE for all fish from 6 replicate cages per treatment.

affecting RMR: Fish reared at low temperature exhibited significantly lower values of RMR (0.253 $mgO_2 \cdot g^{-1} \cdot h^{-1}$) compared with those reared at high temperature (0.447 $mgO_2 \cdot g^{-1} \cdot h^{-1}$). Limiting oxygen concentration—the oxygen concentration at which RMR is limited—is the other component of MMS. Values of LOCr for those fish in the different treatments (1.18 – 2.77 mg/L) did not differ significantly at $P < 0.05$, either between temperature or feed energy levels, or with the interaction of temperature and feed energy.

Modeling

Simulations of Experiment I using Ecophys.Fish resulted in an adequate match (i.e. simulated results within a 95 % confidence interval of observed results) for growth rate, RMR, and MMS for all treatments except Low Temp, LE (Figure 4.3). The lowest MMSO value required to achieve adequate fit (0.110) was obtained for fish in the low-temperature treatment consuming the LE diet; whereas, fish in the high temperature treatment consuming the HE diet required the largest value of MMSO, 0.333 (Table 4.3). The lowest value of the required Winberg-adjustment was observed for fish in the low-temperature treatment consuming the HE diet (1.10), and the highest value was for fish in the high-temperature treatment consuming the HE diet (2.42).

Static Environment - Experiment II: Low Temperature with Low and High Levels of Dissolved Oxygen

Here, temperature was held relatively constant while two levels of DO and feed energy were presented (Table 4.1). Actual levels of DO imposed upon the treatments approximated target values (low DO ~ 27 %; high DO ~ 85%). Growth rate of fish in

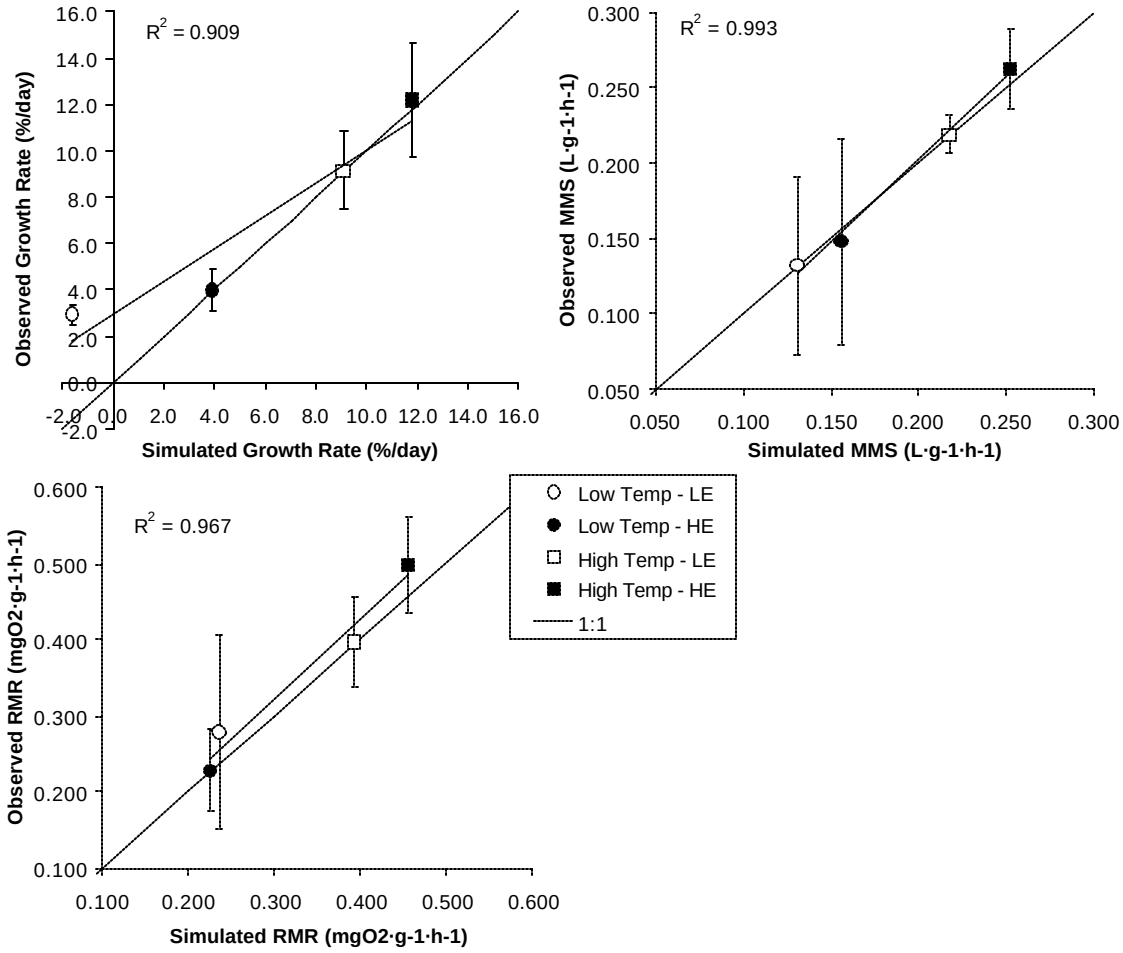

Figure 4.3. Comparison of observed versus Ecophys.Fish-simulated growth rate (%/day, mean +/- 95 % confidence interval); marginal metabolic scope (MMS; $L \cdot g^{-1} \cdot h^{-1}$, mean +/- 95 % confidence interval); and, routine metabolic rate (RMR; $mgO_2 \cdot g^{-1} \cdot h^{-1}$, mean +/- 95 % confidence interval), for red drum in static-environment Experiment I.

Table 4.3. Values of MMSO and Winberg-adjustment necessary to achieve adequate fit between observed and Ecophys.Fish-simulated fish performance for red drum in constant environment experiments.

Static Environment - Experimental Treatment				MMSO	Winberg-adjustment
Exp I	High DO	Low Temp	LE	0.110	2.00
		Low Temp	HE	0.185	1.10
		High Temp	LE	0.275	1.30
		High Temp	HE	0.333	2.42
Exp II	Low Temp	LowDO	LE	0.196	1.32
		LowDO	HE	0.200	1.15
		HighDO	LE	0.085	2.00
		HighDO	HE	0.201	1.05
Exp III	High Temp	LowDO	LE	0.266	1.62
		LowDO	HE	0.242	1.54
		HighDO	LE	0.315	1.72
		HighDO	HE	0.338	2.40

this experiment varied from 1.40 to 7.50 %/day, with significant effects both of DO and feed energy (Table 4.4). Fish exposed to high DO (4.19 %/day) had a greater growth rate than those exposed to low DO (3.28 %/day). As well, feed energy significantly affected growth rate, with those fish consuming the HE diet (5.10 %/day) gaining weight faster than those consuming the LE diet (2.38 %/day). No significant interaction between DO and feed energy was detected for growth rate.

Marginal metabolic scope varied from 0.059 to 0.204 $L \cdot g^{-1} \cdot h^{-1}$. DO and an interaction of DO and feed energy were significant factors; whereas, feed energy had no significant main effects. Contrary to what was observed with growth rate, fish exposed to the low-DO treatment had greater values of MMS (0.160 $L \cdot g^{-1} \cdot h^{-1}$) than those fish exposed to the high-DO treatment (0.114 $L \cdot g^{-1} \cdot h^{-1}$; Figure 4.4), thus suggesting DO acclimation. The interaction effect between DO and feed energy indicates a greater difference in MMS between low-DO and high-DO exposed fish consuming the LE diet compared to those consuming the HE diet.

Routine metabolic rate ranged from 0.099 to 0.480 $mgO_2 \cdot g^{-1} \cdot h^{-1}$, with low-DO-treated fish having higher values of RMR (0.334 $mgO_2 \cdot g^{-1} \cdot h^{-1}$) than high-DO-exposed fish. A marginally significant ($P = 0.054$) effect of feed energy suggested a tendency for those fish consuming the HE diet (0.335 $mgO_2 \cdot g^{-1} \cdot h^{-1}$) to express RMR values that are greater than those of their LE counterparts (0.242 $mgO_2 \cdot g^{-1} \cdot h^{-1}$). Unlike MMS, no interaction between DO and feed energy was detected for RMR. No independent or interactive effects of DO or feed energy on LOCr (1.14 - 2.66 mg/L) were detected.

Table 4.4. Performance of juvenile red drum exposed to constant low temperature at two levels of dissolved oxygen, and with two levels of feed energy-density, during a 4-wk feeding trial (Experiment II).

Environmental Regime		Diet	Growth rate (%/day [a])	Marginal metabolic scope ($L \cdot g^{-1} \cdot h^{-1}$)	Routine metabolic rate ($mgO_2 \cdot g^{-1} \cdot h^{-1}$)	Limiting oxygen concentration (mg/L)
Low Temp	Low DO	LE	2.23	0.169	0.310	1.90
		HE	4.34	0.149	0.367	2.40
	High DO	LE	2.53	0.088	0.174	1.93
		HE	5.85	0.148	0.303	2.07
Analysis of variance, *Pr* > *F* [b] :						
Dissolved Oxygen			0.027 *	0.023 *	0.042 *	0.563
Feed energy			0.000 *	0.210	0.054	0.237
Dissolved Oxygen x Feedenergy			0.127	0.025 *	0.423	0.496
Standard Error			0.177	2.21×10^{-4}	0.002	0.060

[a] Fish initially weighed 2.0 g ± 0.32 (mean ± SD).
[b] Significance [*] probability associated with the *F*-statistic for an analysis of variance of the stated factor; *Pr* > *F*.

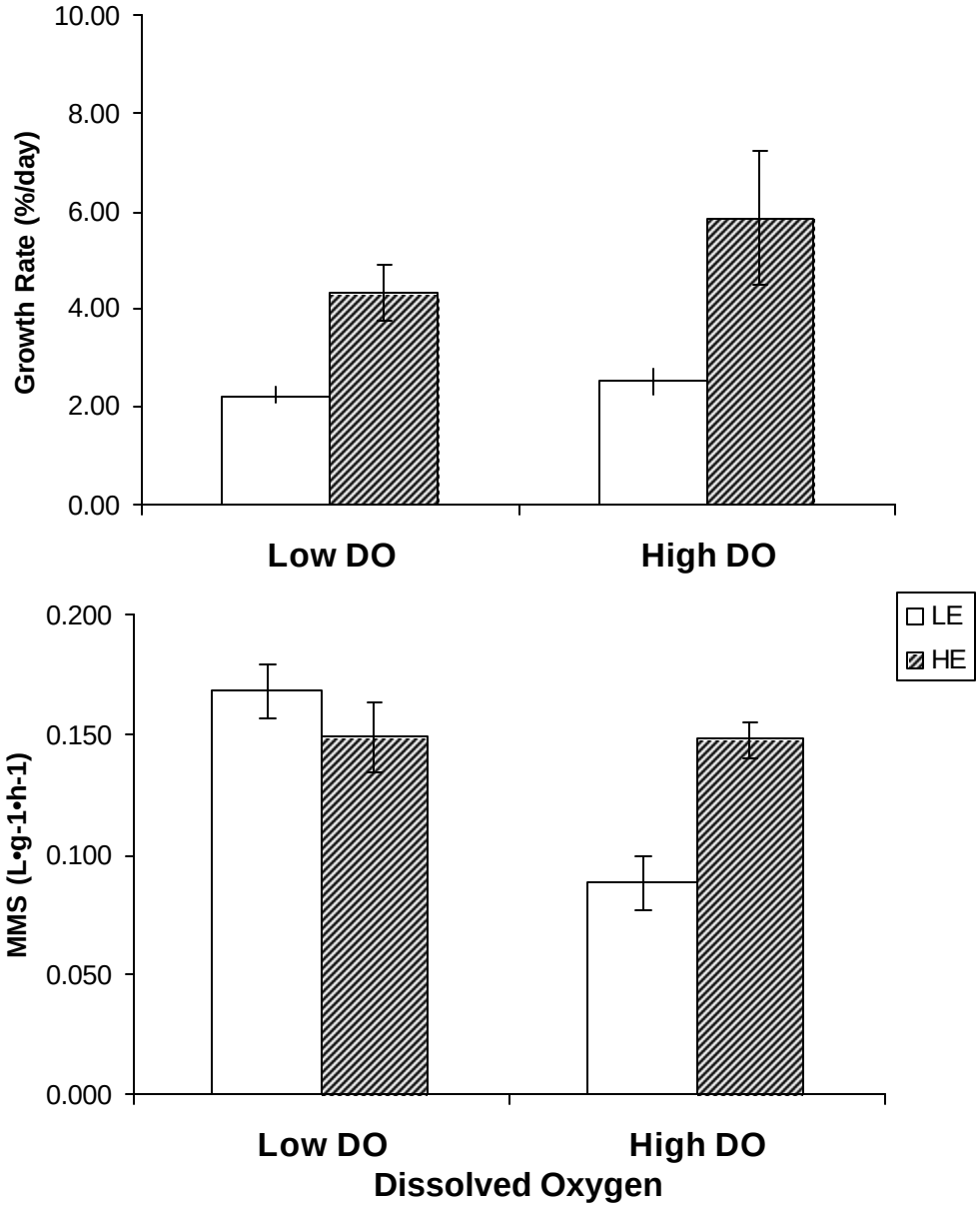

Figure 4.4. Growth rate (%/day) and marginal metabolic scope (MMS; $L \cdot g^{-1} \cdot h^{-1}$) for red drum exposed to low dissolved oxygen (~ 27 % air-saturation) and high dissolved oxygen (~ 85 % air-saturation) while consuming a low-energy diet (LE) or a high-energy diet (HE) in a low-temperature (~ 19 C) environment for a period of 4-wk during Experiment II. Values are presented as mean ± SE for all fish from 6 replicate cages per treatment.

Modeling

As with experiment I, simulations of Experiment II using Ecophys.Fish resulted in an adequate match for growth rate, RMR, and MMS for all treatments except for the High DO, LE treatment (Figure 4.5). This treatment resulted in the lowest MMSO observed (0.085) and a Winberg-adjustment of 2.0 (Table 4.3). The lowest Winberg-adjustment in the three remaining simulations was 1.05 for those fish in the High-DO treatment consuming the HE diet. Also, this treatment exhibited the highest value of MMSO, at 0.201.

Static Environment - Experiment III: High Temperature with Low and High Levels of Dissolved Oxygen

As in experiment II, actual values of achieved DO approximated target levels. Due to the higher temperature, however, values were not identical to those in experiment II (low DO ~ 36 %; high DO ~ 78%). Survival of fish fed the various diets under the warm-water regime while exposed to two levels of DO ranged from 40 to 100 %, with a significant difference attributable to feed energy but not to DO (Table 4.5). Fish in aquaria presented with the HE diet showed a substantially higher rate of survival (86.3 %) over those receiving the LE diet (59.4 %). Growth rate (2.57 – 21.95 %/day) exhibited significant independent and interactive effects of DO and feed energy.

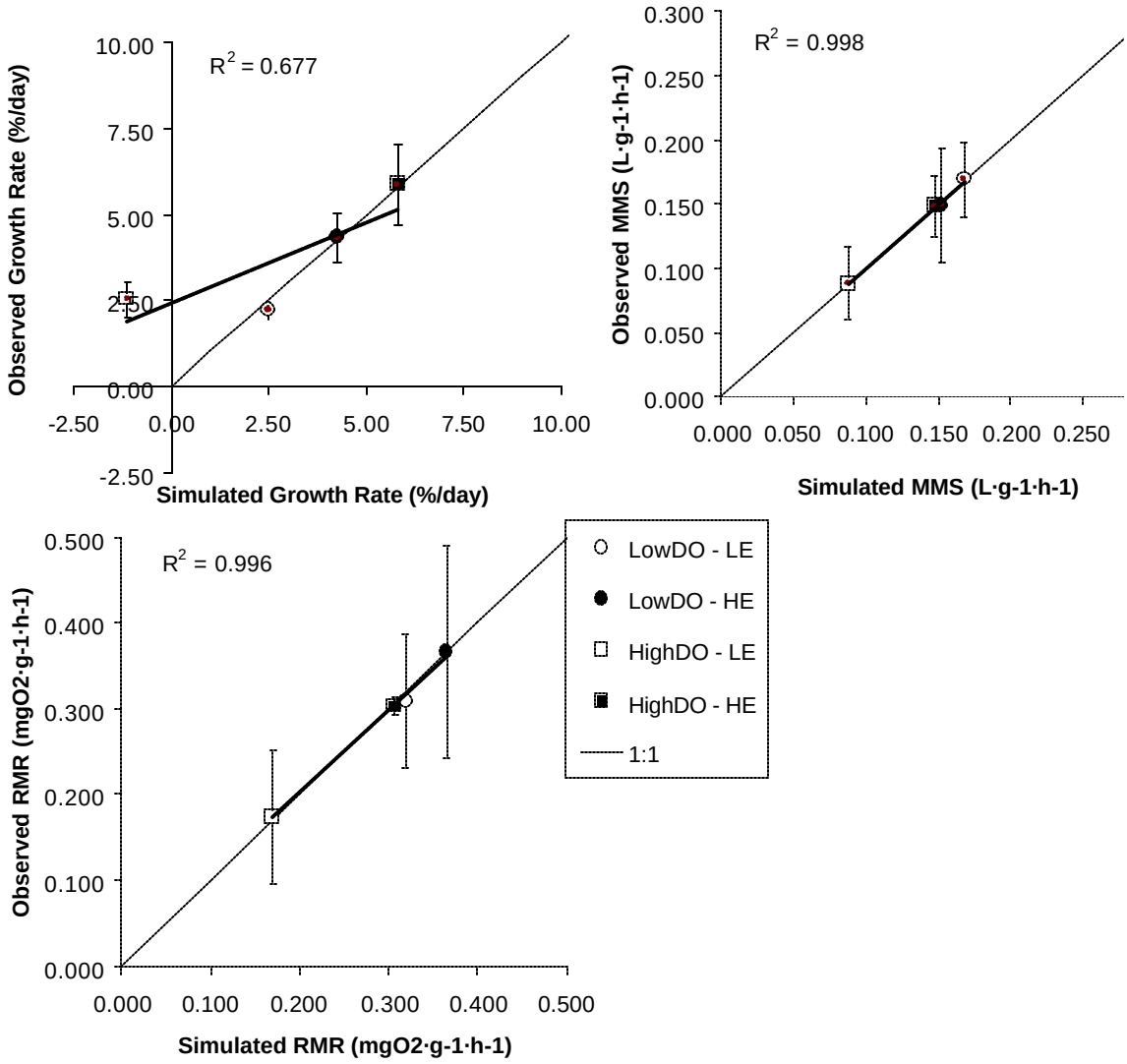

Figure 4.5. Comparison of observed versus Ecophys.Fish-simulated growth rate (%/day, mean +/- 95 % confidence interval); marginal metabolic scope (MMS; $L \cdot g^{-1} \cdot h^{-1}$, mean +/- 95 % confidence interval); and, routine metabolic rate (RMR; $mgO_2 \cdot g^{-1} \cdot h^{-1}$, mean +/- 95 % confidence interval) for red drum in static-environment Experiment II.

Table 4.5. Performance of juvenile red drum exposed to constant high temperature with two levels of dissolved oxygen, and with two levels of feed energy-density, during a 4-wk feeding trial (Experiment III).

Environmental Regime		Diet	Survival (%)	Growth rate (%/day [a])	Marginal metabolic scope ($L \cdot g^{-1} \cdot h^{-1}$)	Routine metabolic rate ($mgO_2 \cdot g^{-1} \cdot h^{-1}$)	Limiting oxygen concentration (mg/L)
High Temp	Low DO	LE	67.50	5.18	0.340	0.462	1.40
		HE	83.75	13.58	0.283	0.453	1.64
	High DO	LE	51.25	6.43	0.275	0.479	1.65
		HE	88.75	20.39	0.240	0.453	1.97
Analysis of variance, *Pr* > *F* [b] :							
Dissolved Oxygen			0.306	0.004 *	0.005 *	0.858	0.225
Feed energy			0.000 *	0.000 *	0.012 *	0.698	0.238
Dissolved Oxygen x Feed energy			0.067	0.030 *	0.480	0.848	0.854
Standard Error			27.73	1.28	2.27×10^{-4}	0.002	0.050

[a] Fish initially weighed 1.1 g ± 0.03 (mean ± SD).
[b] Significance [*] probability associated with the *F*-statistic for an analysis of variance of the stated factor; *Pr* > *F*.

Within the elevated temperature regime of this experiment, a greater growth rate was measured for fish exposed to the high-DO environment (13.41 %/day), compared with that of fish in the low-DO environment (9.38 %/day). Feed energy impacted growth rate, with those fish consuming HE feed growing at a rate considerably greater (16.98 %/day) than LE-consuming fish (5.80 %/day). The significant interaction indicates a greater difference between the growth rates of fish consuming the two diets in the high-DO environment as compared to the low-DO environment.

Metabolically, these fish at elevated temperature demonstrated a significant effect of feed energy as well as of DO, on MMS (0.200 – 0.366 $L \cdot g^{-1} \cdot h^{-1}$). As was observed in Experiment II, low-DO exposed fish exhibited larger values of MMS (0.316 $L \cdot g^{-1} \cdot h^{-1}$), compared with their high-DO counterparts (0.257 $L \cdot g^{-1} \cdot h^{-1}$). These fish demonstrated a sensitivity to feed energy density, with those consuming the LE diet showing greater MMS (0.308 $L \cdot g^{-1} \cdot h^{-1}$) than those consuming the HE diet (0.258 $L \cdot g^{-1} \cdot h^{-1}$; Figure 4.6). Also contrary to Experiment II, no interaction effect of DO and feed energy was detected in Experiment III. Routine metabolic rate varied from 0.340 to 0.614 $mgO_2 \cdot g^{-1} \cdot h^{-1}$, and LOCr ranged from 1.06 to 2.86 mg/L. Perhaps because of this high degree of variation in RMR and LOC, I could not resolve independent or interactive treatment effects.

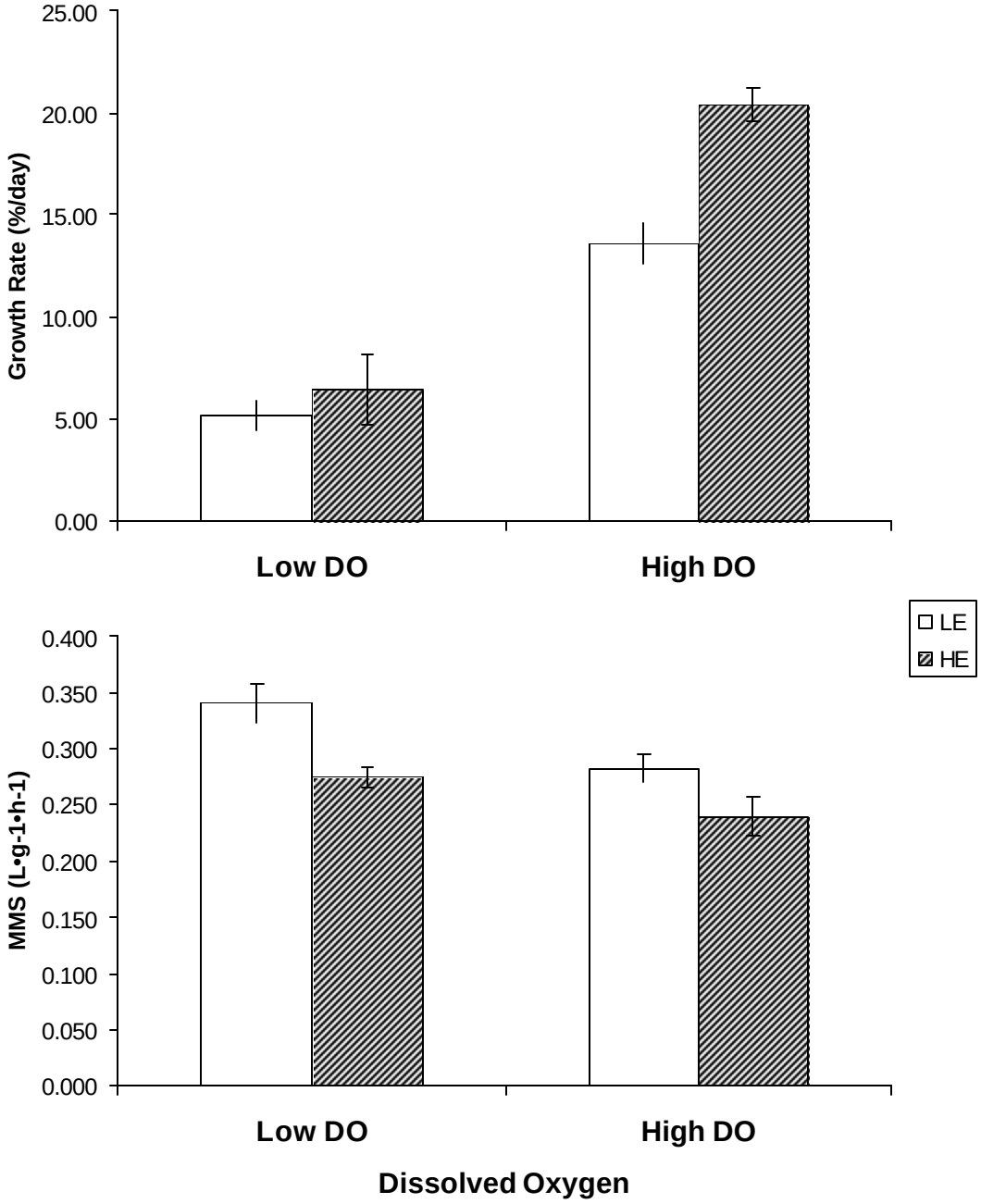

Figure 4.6. Growth rate (%/day) and marginal metabolic scope (MMS; $L \cdot g^{-1} \cdot h^{-1}$) for red drum exposed to low dissolved oxygen (~ 36 % air-saturation) and high dissolved oxygen (~ 78 % air-saturation) while consuming a low-energy diet (LE) or a high-energy diet (HE) in warm water (~ 29 C) environment for a period of 4-wk during Experiment III. Values are presented as mean ± SE for all fish from 6 replicate cages per treatment.

Modeling

Simulations of Experiment III using Ecophys.Fish resulted in an adequate match for growth rate, RMR, and MMS for all treatments (Figure 4.7). The highest value of MMSO (0.338)—both within and across experiments—was observed here in the fish exposed to high DO consuming the HE diet (Table 4.3). Similarly, this treatment resulted in the highest value—again, within and across experiments—of Winberg-adjustment, at 2.40.

Cyclical Environment: Diel Cycling Temperature and Dissolved Oxygen

The diel regimes of environment in cycle experiments I and II were similar (Table 4.1). Temperature ranged from 20.5 to 27.5 C and DO from 25 to 75 % air saturation in experiment I. In experiment II, temperature ranged from 20.0 to 28.0 C and DO from 25 to 76 % air saturation. Growth rate of fish in Experiment I (3.31 – 9.66 %/day) did not differ significantly between feed energy treatments (Table 4.6; Figure 4.8). Metabolically, no effects of feed energy were detected on MMS (0.105 – 0.264 $L \cdot g^{-1} \cdot h^{-1}$), RMR (0.120 – 0.342 $mgO_2 \cdot g^{-1} \cdot h^{-1}$), or LOCr (0.76 – 1.87 mg/L).

The cyclical environmental regime imposed in Experiment II had greater amplitude than that achieved in Experiment I. Fish consuming the HE diet demonstrated increased growth (12.35 %/day) over those on the LE diet (7.57 %/day). Fish consuming the LE diet had higher values of RMR (0.461 $mgO_2 \cdot g^{-1} \cdot h^{-1}$) than those of fish fed the HE diet (0.306 $mgO_2 \cdot g^{-1} \cdot h^{-1}$). No effects of feed energy on MMS or LOCr were resolved in Experiment II.

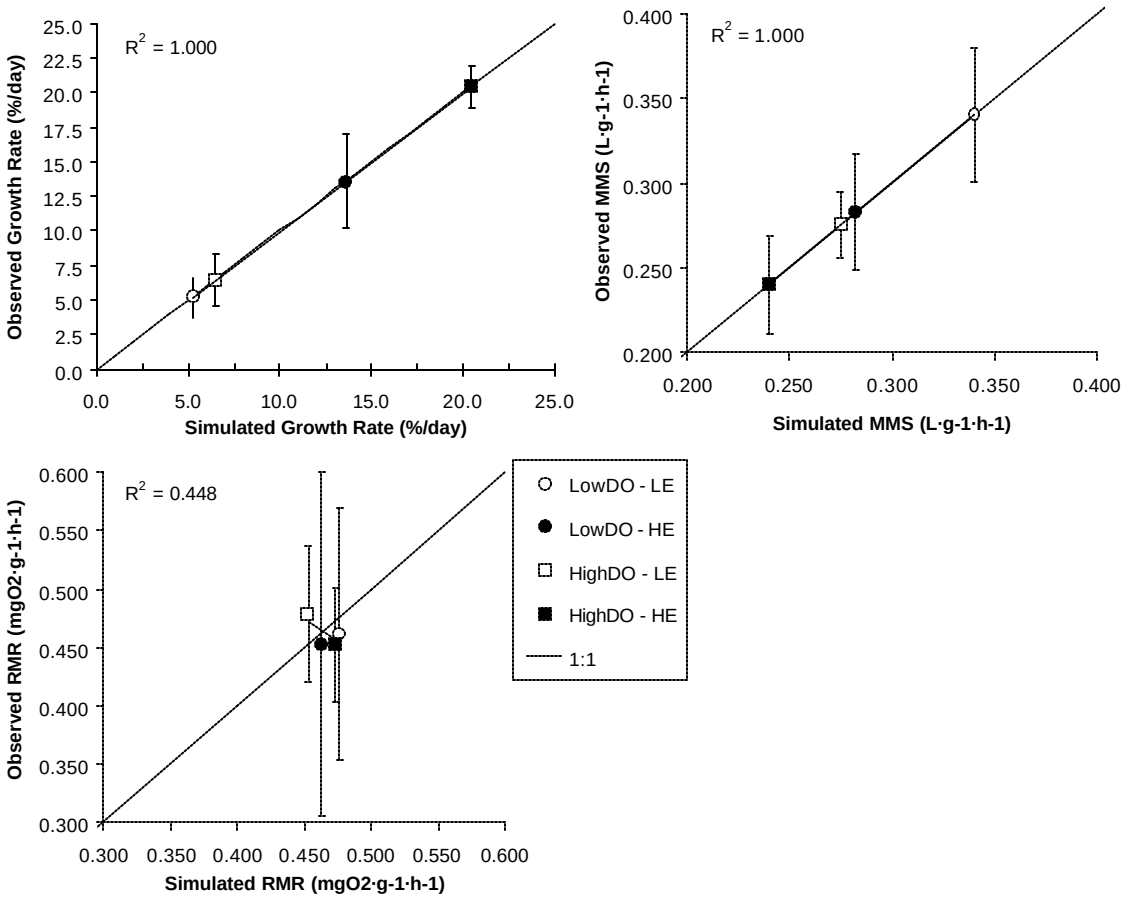

Figure 4.7. Comparison of observed versus Ecophys.Fish-simulated growth rate (%/day, mean +/- 95 % confidence interval); marginal metabolic scope (MMS; $L \cdot g^{-1} \cdot h^{-1}$, mean +/- 95 % confidence interval); and, routine metabolic rate (RMR; $mgO_2 \cdot g^{-1} \cdot h^{-1}$, mean +/- 95 % confidence interval) for red drum in static-environment Experiment III.

Table 4.6. Performance of juvenile red drum exposed to cyclical regimes of temperature and dissolved oxygen while fed the two experimental diets (experiments I and II).

Cyclical Experiment	Diet	Growth rate (%/day[a])	Marginal metabolic scope ($L \cdot g^{-1} \cdot h^{-1}$)	Routine metabolic rate ($mgO_2 \cdot g^{-1} \cdot h^{-1}$)	Limiting oxygen concentration (mg/L)
Experiment I [a]					
	LE	6.62	0.197	0.304	1.54
	HE	5.57	0.185	0.264	1.45
P-value of Feed energy [c]		0.430	0.778	0.631	0.776
Standard Error		1.41	0.034	0.004	0.058
Experiment II [b]					
	LE	7.57	0.329	0.461	1.45
	HE	12.35	0.236	0.306	1.30
Mann-Whitney significance [d]		0.000 *	0.100	0.028 *	0.273

[a] Fish initially weighed 1.6 g ± 0.32 (mean ± SD).
[b] Fish initial average weight 1.0 g (= group weight/number of fish).
[c] Significance [*] probability associated with the t-statistic for a Student's t-test; *Pr* > *t*.
[d] Significance [*] probability associated with non-parametric Mann-Whitney test.

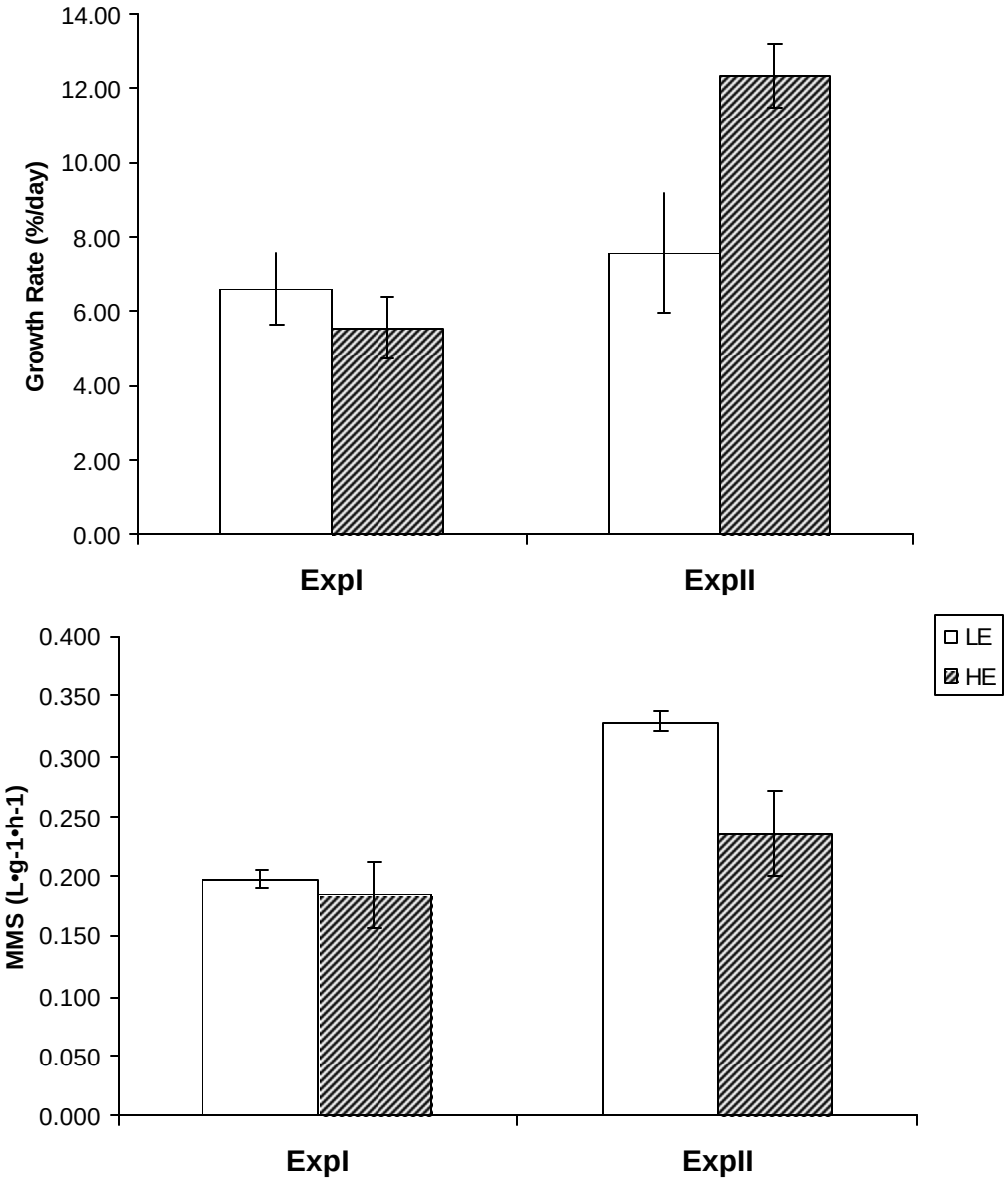

Figure 4.8. Growth rate (%/day) and marginal metabolic scope (MMS; $L \cdot g^{-1} \cdot h^{-1}$) for red drum exposed to cyclical regimes of temperature and dissolved oxygen in two experiments. Values are presented as mean ± SE for all fish from replicate cages.

Modeling

Ecophys.Fish simulations of cyclical-environment experiments I and II resulted in adequate matches for growth rate, RMR, and MMS (Figure 4.9). The lowest value of MMSO necessary to achieve adequate fit was observed in Exp I, with those fish consuming the LE diet. This treatment also had the lowest value of Winberg-adjustment (1.30; Table 4.7a). The largest value of MMSO was observed in Exp II, for fish consuming the LE diet (0.290). Similarly, this treatment exhibited the largest Winberg-adjustment (1.95).

DISCUSSION

Constant Dissolved Oxygen at Two Levels of Temperature

Observed results from the constant-DO experiment were generally consistent with those of previous studies (Neill et al. 2004; Fontaine et al. 2007) in that red drum exposed to lower temperatures exhibited decreased performance. In Experiment I, temperature was a significant factor affecting both growth and metabolism; fish exposed to higher temperature had higher values of growth rate, MMS, and RMR, compared to their counterparts at lower temperatures. Also, a significant effect of feed energy on growth rate was detected in Experiment I; as expected, fish consuming diets lower in energy-density grew at a lower rate compared to those receiving an energetically dense diet. As in Neill et al. (2004) and Fontaine et al. (2007), these effects did not translate into a measurable effect on metabolism as expressed via MMS, RMR, or LOC.

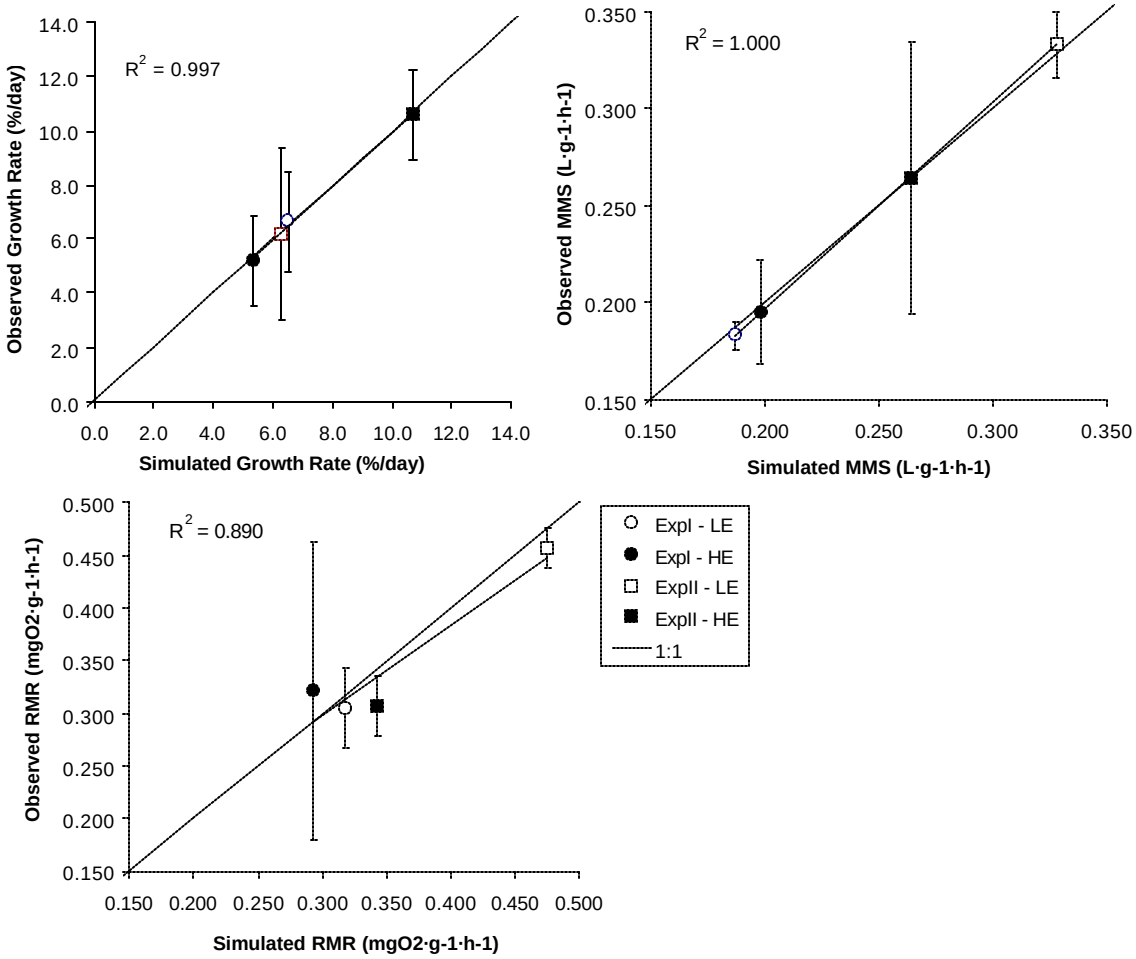

Figure 4.9. Comparison of observed versus Ecophys.Fish-simulated growth rate in cyclical environment experiments I and II (%/day, mean +/- 95 % confidence interval); marginal metabolic scope (MMS; $L \cdot g^{-1} \cdot h^{-1}$, mean +/- 95 % confidence interval); and, routine metabolic rate (RMR; $mgO_2 \cdot g^{-1} \cdot h^{-1}$, mean +/- 95 % confidence interval) for red drum.

Table 4.7. a) Values of MMSO and Winberg-adjustment necessary to achieve adequate fit between observed and Ecophys.Fish-simulated fish performance for red drum in cyclical-environment experiments. b) Resultant values of MMSO and Winberg-adjustment achieved when observed cyclical-environment from experiments I and II altered by 5 % (see text for details). Observed growth rate displayed for ease of comparison with simulated values.

a) Cyclical Environment - Experimental Treatment		MMSO	Winberg-adjustment	Growth Rate (%/day) Observed
Exp I	LE	0.184	1.30	6.64
	HE	0.195	1.50	5.20
Exp II	LE	0.290	1.95	6.18
	HE	0.254	1.92	10.57

b) Environment altered by 5 % from observed		MMSO	Winberg-adjustment	Growth Rate (%/day) Simulated
Exp I	LE	0.178	1.40	5.43
	HE	0.220	1.51	12.50
Exp II	LE	0.286	1.90	6.37
	HE	0.336	2.20	54.36

The greater difference between the metabolic responses of fish consuming the two experimental diets in low-temperature versus high-temperature regimes suggests that those fish in the cooler regime did not have the metabolic capacity to process the additional nutrients and energy available in the HE diet. This finding provides additional evidence in support of temperature-induced changes in limiting factors affecting red drum; specifically, under high-DO conditions, temperature alters limiting factors in red drum such that lower temperatures result in metabolic limitations and higher temperatures in energy-based limitations (Neill et al. 2004; Fontaine et al. 2007).

Modeling results from the constant-environment Experiment I are generally consistent with the findings from the conventional statistical analysis. Ecophys.Fish, however, was unable to accurately replicate the observed growth rate for those fish in the low-temperature, LE treatment. Given the values of MMS and RMR required to achieve a match metabolically, growth was simulated to be negative. By taking a modified or "reverse" approach to model fit I can suggest values of MMS and MMSO that would result in the observed rate of growth. This involves generating values of MMSO based on observed growth rate, *then* generating a value of MMS, as opposed to requiring a match between growth rate and MMS simultaneously. With this procedure, in order for the fish in the low-temperature, LE regime to achieve the observed rate of growth, MMS would have had to be higher than observed (0.239). As well, MMSO would have to be higher than estimated using the nominal method of simulation (0.273). This non-congruence between observed and simulated performance in the same treatment for the following year (see below) suggests not a fluke in the data, but a need to reconsider how

Ecophys.Fish interprets the interactive effects of low temperature and low energy on red drum physiology.

Constant Temperature at Two Levels of Dissolved Oxygen

In an effort to isolate the effects of DO and feed energy on red drum performance, I conducted experiments in which I held temperature relatively constant while the former two factors were manipulated. Using data from previous studies (Vega 2003; Neill et al. 2004; Fontaine et al. 2007) and the *a priori* application of Ecophys.Fish, the levels of environment at which limiting effects would be readily observed were estimated. Also, Ecophys.Fish was used during each experiment to ensure that fish continued to be fed an excess ration.

In the cool-water regime of Experiment II, DO significantly affected both growth and metabolism of juvenile red drum. DO behaved in much the same way as temperature from experiment I; in particular, high levels of DO resulted in greater growth rates of red drum than low levels of DO. As well, feed energy acted on growth such that higher rates were associated with the more energetically-dense diet. The DO treatment affected metabolism such that low levels resulted in significantly greater values of MMS and RMR; DO acclimation may have been providing those fish exposed to low DO conditions with more metabolic capacity.

Fish consuming the energetically dense feeds tended to have higher values of MMSO over their low-energy-consuming counterparts. Ecophys.Fish simulations suggest that in cool-water conditions, feed energy affects metabolic capacity (as measured via MMSO) more so than DO—perhaps due to the ability of the red drum to

readily acclimate to lower levels of DO. Here again, however, using the nominal assumptions, Ecophys.Fish was able to simulate observed performance of all fish in experiment II, *except* those in the low temp, high DO, LE treatment—as was the case in experiment I. As before, using the "reverse fit" modeling, MMS and MMSO had to be higher than observed (0.226 and 0.277 respectively) in order to match growth performance for this treatment.

In Experiment III, fish were exposed to similar low and high levels of DO but in a warm-water regime intended to provide the "optimal" temperature for red drum. Experimental studies and the life history of the red drum suggest maximal growth performance of this fish to be in a temperature range of 25 – 30 C (Arnold 1988; Hopkins, et al. 1988; Robinson, 1988; Lyczkowski-Shultz et al. 1988; Neill 1990). Ecophys.Fish simulations suggested a value of 29 C would provide most favorable growth given the other prescribed abiotic conditions and high-energy feed (Neill et al. 2004). Group survival was found to be higher for those fish consuming the HE diet regardless of DO, suggesting some non-specific health benefit for those individuals on a energy-dense diet.

Under the high-temperature regime of Experiment III, growth and metabolic responses to feed energy and DO were similar, but amplified, as compared to the low-temperature regime of Experiment II; generally, warmer conditions produced higher values of MMSO and Winberg-adjustment, compared with those under cooler conditions. As observed by Fontaine et al. (2007), however, the fish with the lowest growth rates also had the largest MMS. This is considered to be a result of a potentially

confounded relationship between metabolic capacity and body size; metabolic rates tend to be a declining function of body weight (Fry 1947; Brett and Groves 1979; Neill et al. 2004; Fontaine et al. 2007).

Once again, Ecophys.Fish helped to resolve the underlying interactions and relationships via the parameter MMSO. Those fish with the highest rates of growth also exhibited the greatest metabolic capacity. As well, they required the largest value of the Winberg-adjustment, further supporting the supposition that that these fish have an inherently greater capacity for metabolic performance and that they exercised it. Furthermore, these fish had the highest rates of survival. Together, these results imply great performance enhancements resulting from the nutritional and environmental regime of warm water, high DO, and an energetically dense feed. With DO not serving as a limiting factor, the warm-water environment allowed for maximal exploitation of nutrition for growth and metabolism.

Conversely, the response of those fish exposed to the "sub-optimal" situation of low DO in a high-temperature environment is highly informative as well. The results obtained confirmed that higher temperatures tend to elevate physiological rates of red drum. Furthermore, when exposed to low DO—and high temperature—red drum that consumed a high-energy diet had lower values of MMSO than their counterparts on a low-energy diet under similar environmental conditions. In this way, increased feed energy appears to have an oppressive effect on metabolic capacity in a warm-water environment when DO is reduced to limiting levels. Alternatively stated, if DO is limiting metabolic performance (i.e. under low-DO conditions), further restricting

resources by presenting a low-energy diet may actually benefit overall metabolic capacity of red drum. In this manner, MMSO is able to serve as an indicator of *potential* performance. Presumably, if those fish exposed to a regime of warm water, low DO, and low energy were switched to a regime of warm water, high DO, and high energy they would have the available metabolic capacity to begin exploiting the improved conditions immediately.

The combination of Ecophys.Fish and traditional statistics aids in the interpretation of the experiment as a whole. Previous work by Clark (2003) under similar laboratory conditions demonstrated a positive correlation between MMSO and the Winberg-adjustment for red drum. When a similar analysis is performed on the data from the static environment experiments in this study, the responses are very similar to those obtained by Clark (2003) (Figure 4.10a and b).

When the data from the static environment experiments in this study are combined, the fish exposed to the high-temperature regime—regardless of feed energy density or level of DO—demonstrate almost the identical response as measured by Clark (2003) for "healthy" red drum:

$$MMSO_{(Clark\ 2003;\ healthy\ red\ drum)} = 0.07*Winberg\text{-}adjustment + 0.18;$$

and, in this study, fish exposed to high-temperature conditions—regardless of feed energy density or level of DO—

$$MMSO_{high\ temp} = 0.07*Winberg\text{-}adjustment + 0.17.$$

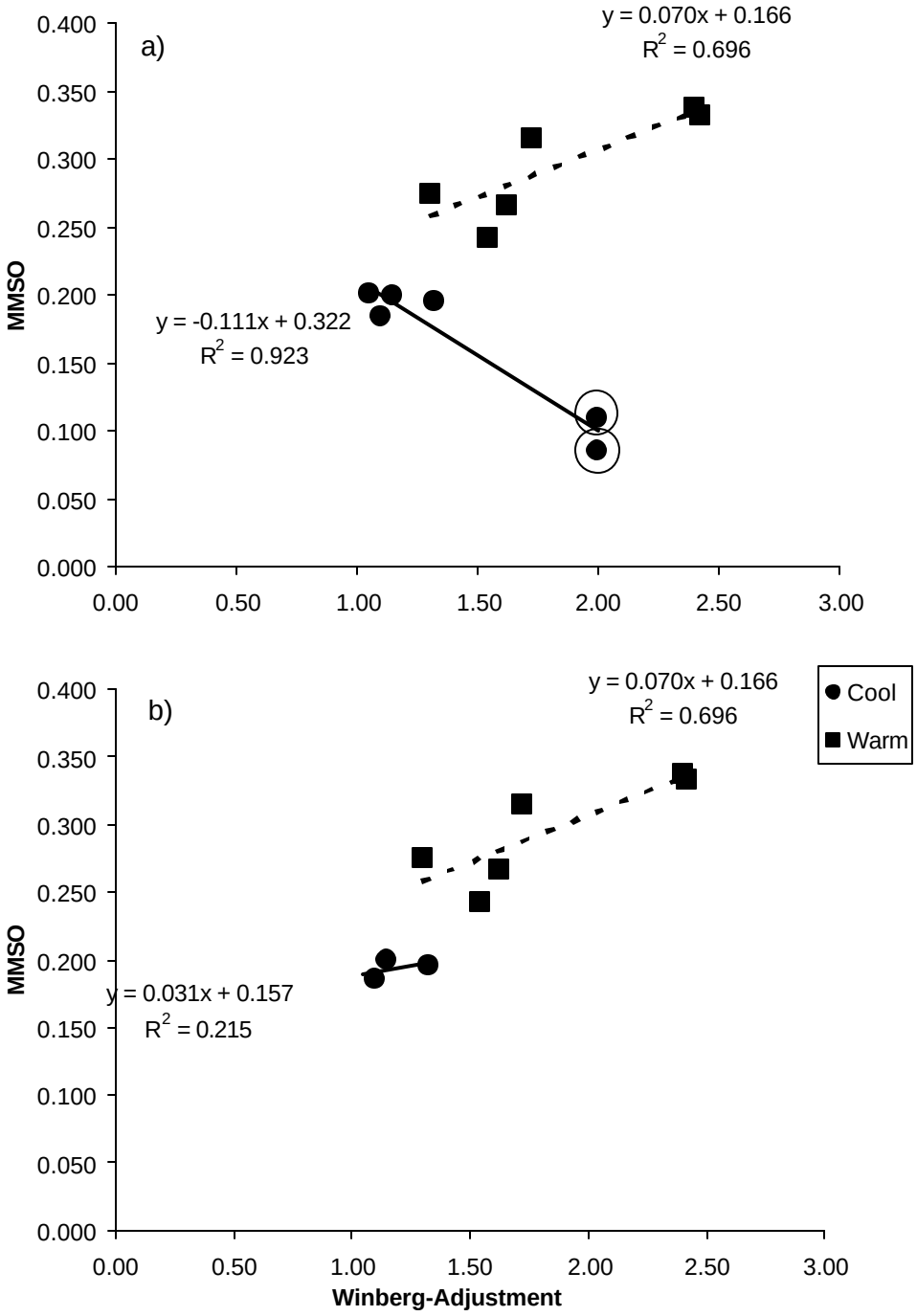

Figure 4.10. MMSO versus Winberg-adjustment for all fish in the static-environment experiments a) with Low Temp, LE points (circled) and b) without Low Temp, LE points.

When all the data are considered for those fish exposed to low-temperature conditions—again, regardless of feed energy-density of level of DO—a strong negative correlation results, due to the joint response of low-temperature, high-DO, and LE diet fish in experiment I and II (Figure 4.10a). When these responses are excluded from the analysis (Figure 4.10b) the relationship dissolves. Similarly, Clark (2003) demonstrated a null-relationship between MMSO and Winberg-adjustment in "sick" red drum; that is, in those that had become ill due to an infection with lymphocystis.

Cycling Temperature and Cycling Dissolved Oxygen

Two repetitions of the cycling-environment experiment were performed to assess potential effects of diel variation in temperature and DO on performance of red drum. As was observed in the static-environment experiments, fish consuming the HE diet exhibited substantially greater growth compared to fish consuming the LE diet in experiment II but not experiment I. Those fish consuming the HE diet in experiment II, grew nearly three times as fast as those given the LE diet. Nevertheless, dietary energy had little apparent effect on metabolism, except where RMR was greater for fish consuming the LE diet in experiment II. No commensurate effect on MMS was measured despite this finding. As previously suggested, that slower-growing fish had higher rates of RMR is probably a result of the tendency for metabolic rate to be a declining function of body size (Fry 1947; Brett and Groves 1979; Neill et al. 2004; Fontaine et al. 2007).

The results from cycle experiment I are similar to those obtained by Perez-Dominguez (2004) with larval red drum. Environmentally realistic temperature and DO

cycles may impart benefits realized in the form of compensatory acclimation and/or increased tolerance for environmental variability, but not necessarily improved growth. In the case of the cyclic experiments in the present study, however, the inconsistency of significant effects between cycle experiments I and II is conspicuous given that feed energy was a significant factor in all the static environment experiments. Behavioral factors may have contributed to this lack of a significant effect. Measures were taken to ensure that fish were presented with an amount of food well beyond that which they were able to consume in a 24-h period. As well, feeding rates were adjusted such that those fish receiving the LE diet were presented with an equivalent amount of food, on a dry-matter basis, as those fish on the HE diet (Fontaine et al. 2007). Stocking density, however, differed between experiment I and the other experiments; one fish per cage was stocked in experiment I whereas multiple individuals were stocked in the other experiments.

Previous work with red drum by Vega (2003) did not indicate significant differences in growth rate for fish reared individually versus those in groups housed in 1-m soft-mesh cages. The plastic 19-L cages used in this study, however, may have provided too-isolated a confinement for the individual experimental fish; sensory communication with neighboring fish may have been so severely restricted that normal socio-behavioral cues could not be effective. No formal comparison of individual vs. group feeding habits has been conducted with red drum; however, a strong social element has been associated with foraging behavior in guppies (Kendal et al. 2004) and is apparent for juvenile red drum in aquaculture. As well, evidence suggests that low—

but not as low as one individual per cage—stocking densities tend to be optimal to ensure continued feeding, growth, and overall health of many fish species (Kristiansen et al. 2004).

For the remaining data, the results from the cyclical-environment experiments tend to be in agreement with those from the constant environment experiments. That is, increased feed energy was correlated with greater growth rate, which suggests growth was behaving as an energy-limited response under both cyclical and constant environmental regimes. The lack of response in MMS emphasizes that cyclical regimes of temperature and DO as presented did not hinder—but neither did they greatly enhance—metabolic capacity.

Previous work with Ecophys.Fish suggested that cyclical regimes of temperature and DO might result in substantially greater rates of growth and metabolic capacity of juvenile red drum over static environments, even those at "optimal" levels (Neill et al. 2004). While I cannot statistically compare performance results under the cyclical environments with those under the constant environments, the data from this study do *not* support the hypothesis of performance enhancement as a result of the cyclical regime. Nevertheless, the observed patterns of growth for fish in the cyclic-environment experiments—i.e., absence of out-performance—were generally accountable under Ecophys.Fish, properly equipped with values of MMSO and the Winberg-adjustment consistent with the empirical respirometry data. So, it is entirely possible that cyclic environmental regimes cause shifts in MMSO and the Winberg-adjustment that were not anticipated by Neill et al. (2004).

Another possibility is that I did not observe the response predicted by Neill et al. (2004) due to my inability to induce the requisite environmental regime. Logistical constraints limited the range and pattern of environment I was capable of presenting. Performance enhancement may be optimized by exposure to diel cycles with greater amplitude, as simulated by Neill et al. (2004). Once again, Ecophys.Fish provides a platform with which to explore such possibilities. By inputting modestly increased amplitudes of the cycled environmental variables—specifically, temperature and DO, each by 10 %—I simulated how fish performance *might* have differed under slight variants of the observed environmental regimes. This was accomplished by increasing the upper values and decreasing the lower values of temperature and DO by 5 % each, then adjusting MMSO and the Winberg adjustment until MMS simulated matched MMS observed. The resultant output (Table 4.7b) suggests that even with a moderate 5 % deviation from observed temperature and DO at each extreme, fish growth and metabolic performance *potentially* would have been much improved. Under the model, the greatest overall physiological benefits are realized for fish consuming an energy-dense diet. Future work to explore and evaluate improved growth performance under cyclical conditions is warranted and could help to elucidate such a relationship.

Other possibilities for the varying performance responses among the experiments in this study are seasonal differences and/or location of the source hatchery. Fish from constant experiment I and cycle experiment I were hatched and initially reared at the TPWD – MDC facility in Flower Bluff, TX, and obtained in September 2004; those used in constant experiment II were hatched and initially reared at TPWD – MDC and

obtained in May 2005; fish from constant experiment III and cycle experiment II were hatched and initially reared at the TPWD – Sea Center Facility in Lake Jackson, TX, and obtained in July 2005. While the MDC and SCT facilities utilize similar rearing procedures and have equally rigorous standards for quality control/quality assurance (Dr. R.R. Vega, personal communication), the natural variation in numerous factors, related either to location or season/year, could influence fish performance over time. At what is perhaps the most fundamental level, genetic differences could be a cause as genetics studies of red drum populations in the Gulf of Mexico indicate a certain degree of genetic divergence with increasing distance between bay systems (Gold 1999; Gold and Turner 2002).

The findings of this study support the idea that the red drum is a highly adaptable, euryhaline species. This adaptability is critical to the ability of this fish to survive in variable conditions characteristic of its native estuarine habitat. Under hatchery or aquacultural conditions, managers may mitigate losses due to ephemeral periods of poor environment and nutrition or manipulate these conditions to fully exploit the robust energy-processing and metabolic capabilities of juvenile red drum.

CHAPTER V

EFFECTS OF DIETARY AND ENVIRONMENTAL MANIPULATION ON PERFORMANCE OF HATCHERY-REARED JUVENILE RED DRUM IN COASTAL TEXAS PONDS

SYNOPSIS

Two cage studies with juvenile red drum (*Sciaenops ocellatus*) were conducted in culture ponds at the CCA/CPL Marine Development Center, a facility operated by Texas Parks and Wildlife Department in Corpus Christi, Texas. The objective of the studies was to evaluate hypotheses regarding the effects of specific dietary and environmental manipulations on red drum performance. In the first study, the commercial prebiotic GroBiotic®A was added as a supplement to two experimental diets of differing energetic density—low energy (LE; ~ 4.1 kJ/g) and high energy (HE; ~ 15.9 kJ/g)—in an effort evaluate potential effects on survival, growth rate, routine metabolic rate (RMR), limiting oxygen concentration (LOC), and marginal metabolic scope (MMS). In the second study, gaseous oxygen was administered during evening hours to the water in cages containing red drum fed the LE or HE feeds, to assess joint impacts of feed energy density and oxygen-supplementation on these same indicators of fish performance. Results indicated a strong positive effect of feed energy density on growth of red drum across all treatments. The dietary supplement utilized here tended to decrease survival of caged juvenile red drum. Simulation modeling with Ecophys.Fish suggests that fish receiving the LE diet were consuming a diet higher in caloric density than intended. Red drum consuming the HE diet under the oxygen-supplementation

treatment grew significantly faster than their counterparts not receiving additional oxygen. However, modeling supports the findings that the observed nocturnal levels of DO did not limit either growth or metabolic capacity.

INTRODUCTION

A growing human population and increasing demand for protein-rich fish as food have put pressure on fisheries resources. Over-fishing has resulted in depletion of coastal fisheries resources and threatened the viability of various fish populations world-wide (Rutledge 1989; McEachron and Daniels 1995; McEachron et al. 1998; Blaxter 2000; Hong and Zhang 2003; Liao et al. 2003). Despite concerns about genetic and ecosystem modification, stock enhancement and related aquacultural activities are increasingly being recognized and relied upon for their potential to increase and sustain fisheries (McEachron et al. 1995; Tringali and Bert 1998; Masuda and Tsukamoto 1998; Tidwell and Allen 2001; Mustafa 2003; Vega et al. 2003). Development of novel and effective feed technologies and methodologies as well as continued efforts to better understand the effects of environment on the performance of a target species remain critical components of a successful stock-enhancement strategy (Blankenship and Leber 1995; Lee 1997; Serafy et al. 1999; Fushimi 2001)

In recent years, one of the most important breakthroughs in fish-nutrition research is discovery that a variety of natural and synthetic compounds can confer benefits to growth, immunity and other aspects of performance in cultured fishes, such benefits extending well beyond those accountable under conventional bioenergetics models (Sakai 1999; Gatlin 2002a; Li and Gatlin 2006). One group of compounds

known as "prebiotics" may hold the potential to enhance survival and performance of fish aquacultured for seafood production as well as for release into the wild for stock enhancement. Prebiotics alter intestinal conditions to favor certain beneficial bacteria such as *Lactobacillus* sp. and *Bifidobacter* sp.. Brewers yeast (*Saccharomyces cerevisiae*), a rich source of immunomodulatory compounds such as ß-glucans and nucleotides, has been reported to enhance immunity and disease resistance in several economically important warmwater fish species such as hybrid striped bass (Li and Gatlin 2003; Li et al. 2004; Li and Gatlin 2006) and gilthead sea bream *Sparus aurata* (Ortuño et al. 2002; Borda et al. 2003). GroBiotic® A—a commercially available prebiotic—contains a mixture of partially autolyzed brewer's yeast, dairy products, and dried fermentation products. This supplement has been found to enhance survivability, disease resistance, and growth in hybrid striped bass *Morone chrysops* × *M. saxatilis* (Li and Gatlin 2003; Li et al. 2004; Li and Gatlin 2006); however, results from preliminary laboratory-based studies evaluating these individual compounds on red drum have not demonstrated consistent effects (Li et al. 2005). Nevertheless, these findings did prompt interest in evaluating this dietary supplement to potentially improve performance and survival of red drum in the context of a hatchery-pond environment. In the first stage of this study, I conducted a dietary-supplementation experiment to evaluate effects of GroBiotic® A (GroBio) on growth and metabolic performance of caged juvenile red drum consuming diets with different levels of available energy.

Previous laboratory and pond studies, and simulation-modeling studies with red drum have provided intriguing suggestions of dramatic effects of feed energy and

environmental variation on red drum survival, growth, and metabolism (Perez-Dominguez and Holt 2002; Neill et al. 2004; Fontaine et al. 2007). These studies have supported an energy/metabolism trade-off hypothesis which suggests that red drum growth in aquaculture, where the fish normally are fed energy-dense prepared feeds, may be limited by available metabolic scope—the metabolic capacity to process available dietary energy. Under this hypothesis, maximal exploitation of the nutritive benefit of a feed is achieved through matching complementarity of available feed energy and metabolic capacity; thus, there exists a "tradeoff" between available dietary energy and the metabolic capacity to process this energy. Through directed manipulation of environmental conditions—namely, dissolved oxygen—I hoped to maximize the metabolic capacity of red drum. In the oxygen-supplementation portion of this study, I expected to observe improved growth for fish exposed to O_2-enriched conditions while consuming a diet high in digestible energy, compared with their counterparts not receiving oxygen-supplementation or those receiving an energy-poor diet.

METHODS

The four experimental diets were prepared and stored following Fontaine et al. (2007). Briefly, the basal high energy diet (HEb) was a highly nutritious, dry pelleted-feed originally developed for research purposes and designed to contain 10% moisture, 40% protein, 10% lipid and an estimated 3.5 kcal DE/g on a dry-weight basis. This formulation met or exceeded all known nutritional requirements of red drum and most other warmwater fishes (Robinson 1988; NRC 1993; McGoogan and Gatlin 1998; Gatlin 2002b, Webb and Gatlin 2003, Li et al. 2005). The basal low energy diet (LEb) was

designed to contrast with the HEb diet and to more closely resemble the red drum's natural forage with regard to moisture, protein, lipid, and energy content. This low-energy alternative was formulated to contain 80% moisture, 12% protein, 2% lipid and an estimated 0.8 kcal DE/g on a fresh-weight basis. Additionally, I evaluated each basal diet supplemented with the commercial prebiotic GroBiotic®A (LEg and HEg) added at 2% dry-weight (International Ingredient Corporation, St. Louis, MO, USA).

Red drum larvae were produced from captive broodfish maintained at CCA/CPL Marine Development Center (MDC), a TPWD-operated facility in Corpus Christi, TX; then, the larvae were cultured to the early juvenile stage in ponds either at MDC or at a second TPWD-operated facility, the Perry R. Bass Marine Fisheries Research Station (PRB), near Palacios, TX. Fish for the first experiment originated from the MDC ponds; whereas, those for the second experiment were obtained from PRB ponds. Between the first and second pond experiments, pond production of red drum juveniles was shifted from MDC ponds to those at PRB, because hot and arid weather had caused environmental conditions in the ponds at MDC to become too severe (salinity ~ 50 ppt, temperature ~ 32 C; TPWD unpublished data) for maximum yields. While utilizing fish with two different culture histories is not ideal for scientific research, it is a realistic scenario for hatchery managers given the variable environmental, biological, and nutritional conditions encountered in the dynamic bay and estuary ecosystems of the Gulf of Mexico.

Fish were reared according to TPWD protocols in their respective ponds, until the normal point of harvest and release—when an approximate mean weight of 0.5 g is

achieved or when natural forage has been exhausted. Upon harvesting, a portion of the fish was transferred to a pair of 0.4-ha, plastic-lined ponds at MDC; fish from PRB were hauled to MDC in an oxygenated TPWD tank-trailer (trip duration, about 2 h), according to normal protocols. Randomly sampled fish from the group were placed into submerged 1 m x 1 m (height x diameter) cages with 3-mm mesh (Vega 2003) within each of the two replicate MDC ponds. Each cage had been attached to and set on top of concrete cinderblocks (approximately 20x20x41 cm) to position them within the pond, elevate them from the pond bottom, and facilitate water exchange. In each duplicate pond, four individual fish with an average weight of 0.51 ± 0.16 g (mean ± SD) were stocked into each triplicate cage in the first experiment (feed energy density and prebiotic supplementation—henceforth, the "feed experiment"), and ten individuals (0.40 ± 0.17 g) were stocked into each cage in the second experiment (feed energy density and oxygen supplementation—henceforth, the "feed-oxygen experiment"). Mean and standard deviation of fish initial weight was estimated by individually weighing a random sample of 200 fish. To ensure that each hatchery pond contained a concentration of biomass similar to that typical during actual operations, free-swimming individuals of the same size distribution were stocked into each pond at a density of ~23,000 fish/0.4 ha in the feed study and ~34,000 fish/0.4 ha in the feed-oxygen study; throughout the duration of the experiment the free-swimmers were fed a commercial diet at a rate of ~ 20 % of estimated biomass/day.

Prior to harvest and stocking into the enclosures, all fish were consuming the natural forage available in their respective culture ponds as a consequence of TPWD's

standard pond fertilization regime (Colura et al. 1976; Colura 1990; Vega et al. 1995; Vega 2003). The growth-trial phase of each experiment was initiated when fish were placed into the cages and first presented with the feed or feed-oxygen treatments. Fish were fed—in excess—the appropriate experimental diet twice daily via a 152-mm diameter access port on the top of each cage. Fish in the feed experiment were fed low-energy or high-energy formulations of the experimental feed (LE or HE), either with or without prebiotic supplementation (LEg, HEg; or, LEb, HEb), for a period of 4.5 weeks. Fish in the feed-oxygen experiment were provided with the basal formulations of one or the other feeds (LEb and HEb) for a period of 5.7 weeks.

To adjust feeding rates for fish growth, I used the simulation model Ecophys.Fish (Neill et al. 2004; Fontaine et al. 2007) in conjunction with weekly samples of the fish free-swimming in the pond. The top of each cage had been equipped with a fine-mesh (35 micron) funnel secured with twine to prevent escape of fish while allowing easy access for stocking of fish and daily feedings. Water-quality parameters in the vicinity of the cages were monitored throughout each experiment. Data-logging environmental probes (YSI 6000-series) were deployed in each replicate pond and set to measure and record hourly values of temperature (C), salinity (ppt), conductivity (mS/cm), DO (mg/L), and pH for the duration of each experiment. The data were recovered at the termination of each experiment and incorporated into Ecophys.Fish for further analysis (Figures 5.1 and 5.2). Following TPWD protocol, water-quality parameters also were measured near the cages with a hand-held meter (YSI 85) at 04:00, 15:00, and 22:00 H each day. Also in accordance with TPWD procedures, paddlewheels were operated

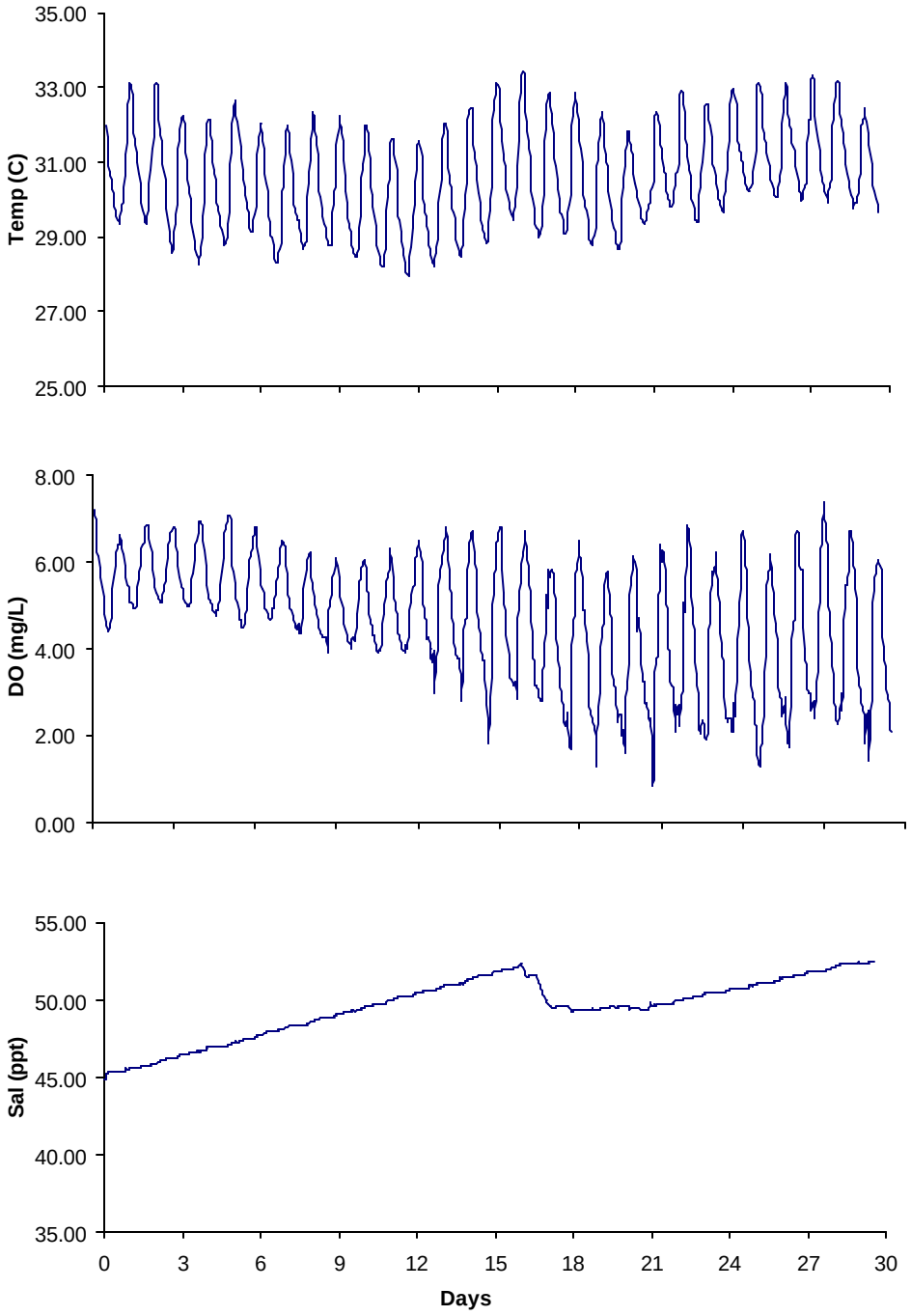

Figure 5.1. Temperature (C), dissolved-oxygen concentration (mg/L), and salinity (ppt) as recorded hourly for 30 days during the feed experiment. Note: Values displayed represent medians for two replicate ponds.

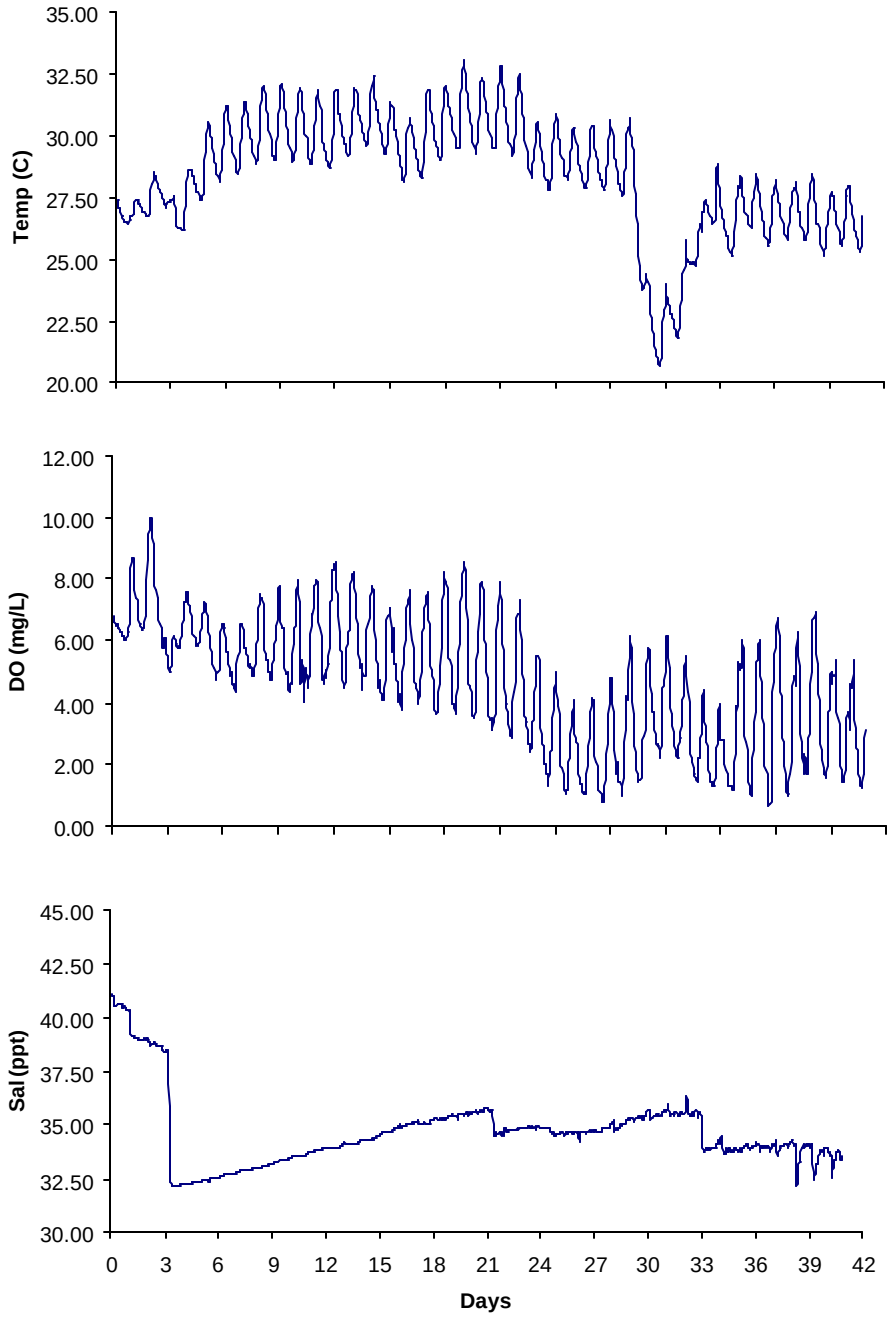

Figure 5.2. Temperature (C), dissolved-oxygen concentration (mg/L), and salinity (ppt) as recorded hourly for 41 days during the feed-oxygen experiment. Note: Values displayed represent medians for two replicate ponds.

overnight to prevent anoxic conditions from developing in the ponds. A random subset of cages in the feed- oxygen experiment contained air stones supplied with compressed oxygen from tanks, to minimize the overnight decline in dissolved oxygen (DO) caused by respiration of the biota. Cages receiving oxygen-supplementation will be referred to as O_2+ while those exposed to ambient oxygen levels are designated by AmbO_2. In the O_2+ cages, oxygen flow was turned-on at the evening feeding (~17:00) and turned-off at the morning feeding (~07:00); oxygen tanks were replaced as necessary.

At the termination of both experiments, whole cages were removed in order to sample the individual fish for survival, growth, and metabolic performance. Survival was based on the number of individuals stocked into a cage compared to the number remaining alive when that cage was harvested. Missing individuals were assumed to have died. Growth rate was calculated from the estimated average initial fish weight, final observed fish weight, and the number of days spent in the cage. Fish were allowed to fast for 24 hours prior to placement into respirometers. Remaining individuals not used in respirometry were euthanized according to TPWD protocol. Metabolic performance was measured following Fontaine et al. (2007) using automated routine respirometry. Specifically, I obtained for each fish a median value of routine metabolic rate as adjusted for biological and chemical oxygen demand ($mgO_2 \cdot g^{-1} \cdot h^{-1}$), limiting oxygen concentration (mg/L), and their ratio, marginal metabolic scope (MMS; $L \cdot g^{-1} \cdot h^{-1}$). Serving as an index of overall metabolic capacity, MMS was calculated for each fish based on the median values of RMR and LOC that were deemed

acceptable for the 18-22 h respirometry trial with a given fish (Springer and Neill 1988; Neill and Bryan 1991; Fontaine et al. 2007). Respirometry was conducted on-site, at an indoor facility at the MDC, at a temperature of ~ 25 C. Throughout the duration of sampling, fish were exposed to water only from their respective ponds to ensure consistency of water chemistry. Continuous (24-h light, 0-h dark) fluorescent lighting was used during respirometry. Final fish weight was obtained immediately prior to placement within the respirometer chamber. After ~ 20 hours of respirometric measurements, fish were removed from the chambers, euthanized, and preserved in cold storage (-20 C). Immediately following each "fish run," the biological and chemical oxygen demand for that trial was estimated with a 1 hour "blank run" in the corresponding empty respirometer chamber.

Statistical Analysis and Modeling

Differences in treatment means were evaluated via analysis of variance (ANOVA) using SPSS and were considered significant at $P < 0.05$. In both experiments, statistical results are based on the median cage response from each of six replicate cages (three cages from each of the two ponds) per treatment.

Growth and metabolic performance of fish in the feed-oxygen experiment were simulated using the ecophysiological model, Ecophys.Fish. The median environment (temperature, DO, salinity, pH on a 1-h time-step) of the two duplicate hatchery ponds was processed through the model to emerge as growth rate, MMS, and RMR (Vega 2003; Neill et al. 2004; Fontaine et al. 2007). Consistent with the statistical analyses,

comparisons of growth and metabolic date were calculated from the median treatment response.

Modeling assumptions and methodology followed those developed by Neill et al. (2004), Vega (2003), and Fontaine et al. (2007). I assumed that 1) rate of feed presentation was not limiting (unlimited FeedRate); 2) energy density of feed (GEfeed) was 4.10 kJ/g for the LEb and LEg diets, and 15.90 kJ/g for the HEb and HEg diets, with energy digestibilities (FeedDigestibility%) of 74.8 % and 72.9 % for the LE and HE feeds, respectively (Fontaine et al. 2007); and, 3) energy density of modeled fish (GEfish) was initially 4.18 kJ/g (natural weight) but varied thereafter as a function of energy intake relative to the cost of routine metabolism. As described in Fontaine et al. (2007) the latter assumption was implemented by having GEfish increase at 0.3%/day to a maximum of 5.86 kJ/g when the cost of feed-processing metabolism (Ad) exceeded 60 % of routine metabolic rate (Ar), and decrease at 0.3 %/day to a minimum of 3.35 kJ/g when the cost of feed-processing metabolism fell below 60 % of routine metabolic rate.

Nominal iterative simulations proceeded with manipulation only of the MMSO and the Winberg-adjustment parameters until an optimum match between observed and simulated pairs of RMR, MMS, and growth rate were achieved. A match was considered optimum only when the coefficient of determination was greater than 70 % for RMR, MMS, and growth rate, simultaneously. If a match could not be obtained using the nominal model, environmental and nutritional inputs were altered in a systematic and conscientious fashion in order to assess potential reasons for observed

discrepancies. These hypothetical simulations and their assumed parameters are clearly distinguished from results under the nominal model.

RESULTS

In the experiment to evaluate dietary-energy and Grobiotic-A-supplementation effects, survival was calculated from the ratio of the number of fish stocked into each cage to those remaining at harvest and expressed as a percentage. Values averaged 95.8 %, 62.5 %, 87.5 %, and 58.3 % for the LEb, LEg, HEb, and HEg treatments, respectively (Table 5.1). Red drum consuming the Grobiotic-A-supplemented diets had a significantly lower rate of survival compared to their counterparts consuming the basal formulations, ($F = 16.79$, $df = 1$, $P < 0.001$). Neither dietary energy nor the interaction of dietary energy with prebiotic supplementation significantly affected survival at $P < 0.05$ (Figure 5.3). Growth rate averaged 20.8 %/day, 31.4 %/day, 39.9 %/day, and 33.6 %/day for the LEb, LEg, HEb, and HEg treatments, respectively. Those fish consuming the low-energy diets grew significantly less than those maintained on the high-energy diets ($F = 6.15$, $df = 1$, $P < 0.022$). While no main effect of dietary supplementation was detected, the interaction between energy and supplement was marginally significant ($F = 0.25$, $df = 1$, $P < 0.621$; $F = 3.92$, $df = 1$, $P < 0.062$, respectively). This implies a differential response of growth rate to dietary supplementation influenced by the level of energy in the feed, with a tendency for Grobiotic-A to offset the negative impact of low dietary-energy density. None of the metabolic indices of performance—MMS (0.189 to 0.375 $L \cdot g^{-1} \cdot h^{-1}$), RMR (0.202 to 0.687 $mgO_2 \cdot g^{-1} \cdot h^{-1}$), or LOC (0.93 to 2.28 mg/L)—responded significantly either to dietary energy or to dietary supplementation.

Table 5.1. Performance of caged juvenile red drum consuming the various diets in the feed experiment over a 30-day feeding trial.

Feed energy	Supplement	Survival (%)	Growth rate (%/day [a])	Marginal metabolic scope ($L \cdot g^{-1} \cdot h^{-1}$)	Routine metabolic rate ($mgO_2 \cdot g^{-1} \cdot h^{-1}$)	Limiting oxygen concentration (mg/L)
Low Energy	Basal	95.83	20.81	0.320	0.503	1.58
	GroBiotic	62.50	31.42	0.260	0.411	1.62
High Energy	Basal	87.50	39.86	0.277	0.399	1.41
	GroBiotic	58.33	33.55	0.260	0.420	1.61
Analysis of variance, *Pr* > F [b] :						
Feed energy		0.422	0.022 *	0.512	0.557	0.700
Supplement		0.001 *	0.621	0.260	0.659	0.605
Feed energy x Supplement		0.788	0.062	0.526	0.486	0.716
Standard Error		71.23	22.34	8.15×10^{-4}	0.005	0.414

[a] Fish initially weighed 0.51 g ± 0.16 (mean ± SD).
[b] Significance [*] probability associated with the *F*-statistic for an analysis of variance of the stated factor; *Pr* > F.

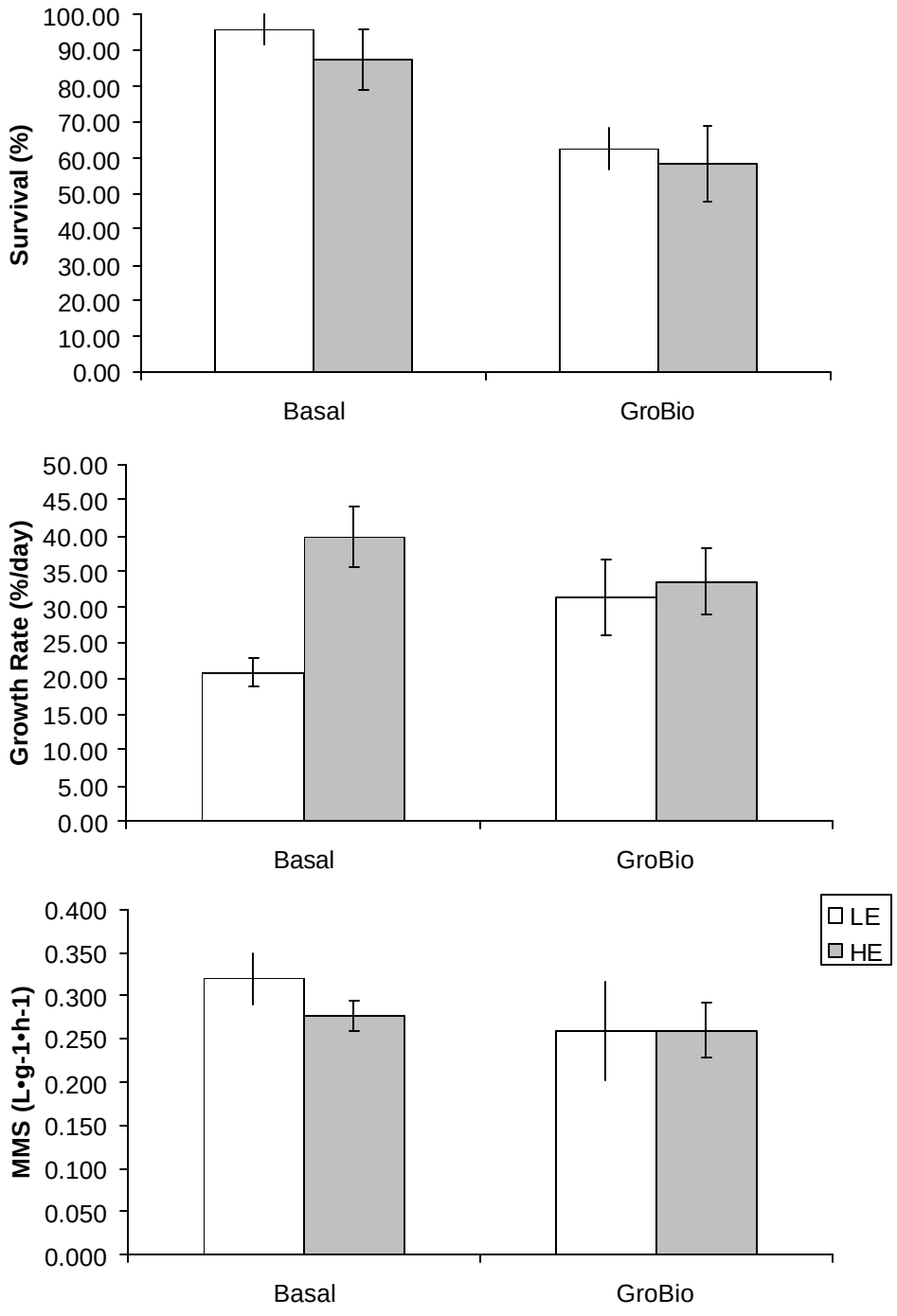

Figure 5.3. Survival (%), growth rate (%/day), and marginal metabolic scope (MMS; ($L \cdot g^{-1} \cdot h^{-1}$) of red drum consuming either a basal formulation or GroBiotic®-supplemented formulation of a low-energy diet (LEg) or a high-energy diet (HEg) during a 30-day dietary-supplementation experiment. Values are presented as mean ± SE for all fish from six replicate cages per treatment. Significance established via analysis of variance at $P < 0.05$.

The feed-oxygen experiment was intended to resolve the differential effects of dietary energy observed in the first experiment, and to test hypothesized effects (Neill et al. 2003; Fontaine et al. 2007) of elevated oxygen on red drum performance. Survival—measured as before—ranged from 10 % to 90 %, with no detected significant effect of dietary energy, O_2 supplementation, or their interaction (Table 5.2). Growth rate averaged 68.9 %/day, 47.2 %/day, 72.6 %/day, and 84.8 %/day for LE AmbO_2, LE O_2+, HE AmbO_2, and HE O_2+, respectively. Statistical testing revealed that red drum consuming the low-energy feed grew significantly less than those on the high-energy alternative ($F = 8.83$, $df = 1$, $P < 0.008$); no significant effect of O_2- supplementation was detected ($F = 0.48$, $df = 1$, $P < 0.507$). However, the interaction of dietary energy and O_2-supplementation was significant ($F = 5.93$, $df = 1$, $P < 0.024$). Thus, O_2-supplementation increased growth rate of fish consuming the HE diet—and decreased growth rate of fish consuming the LE diet (Table 5.2; Figure 5.4).

As was observed in the first experiment, none of the metabolic indices of interest responded differentially to dietary energy at the $P < 0.05$ level, nor did MMS (0.128 to 0.233 $L \cdot g^{-1} \cdot h^{-1}$) exhibit a significant response to O_2 supplementation ($F = 0.11$, $df = 1$, $P < 0.748$). Oxygen supplementation, however, did significantly reduce the RMR of fish in this study ($F = 4.98$, $df = 1$, $P < 0.037$). Similarly, O_2 supplementation showed a marginally significant tendency to reduce LOC ($F = 3.81$, $df = 1$, $P < 0.065$). No significant interaction between dietary energy and O_2 supplementation was detected for any of the metabolic responses.

Table 5.2. Performance of caged juvenile red drum exposed to two levels of oxygen-supplementation while consuming two levels of feed energy during the 41-day feed-oxygen experiment.

Feed energy	Oxygen-Enhancement	Survival (%)	Growth rate (%/day [a])	Marginal metabolic scope ($L \cdot g^{-1} \cdot h^{-1}$)	Routine metabolic rate ($mgO_2 \cdot g^{-1} \cdot h^{-1}$)	Limiting oxygen concentration (mg/L)
Low Energy	$AmbO_2$	41.67	68.86	0.182	0.351	1.97
	O_2+	55.00	47.23	0.173	0.283	1.65
High Energy	$AmbO_2$	50.00	72.59	0.177	0.339	1.91
	O_2+	40.00	84.81	0.171	0.318	1.87
Analysis of variance, *Pr* > F [b] :						
Feed energy		0.692	0.008 *	0.748	0.576	0.385
Oxygen-Enhancement		0.843	0.507	0.469	0.037 *	0.065
Feed energy x Oxygen-Enhancement		0.174	0.024 *	0.906	0.250	0.147
Standard Error		84.03	59.17	0.001	4.69×10^{-4}	0.011

[a] Fish initially weighed 0.40 g ± 0.17 (mean ± SD).
[b] Significance [*] probability associated with the *F*-statistic for an analysis of variance of the stated factor; *Pr* > F.

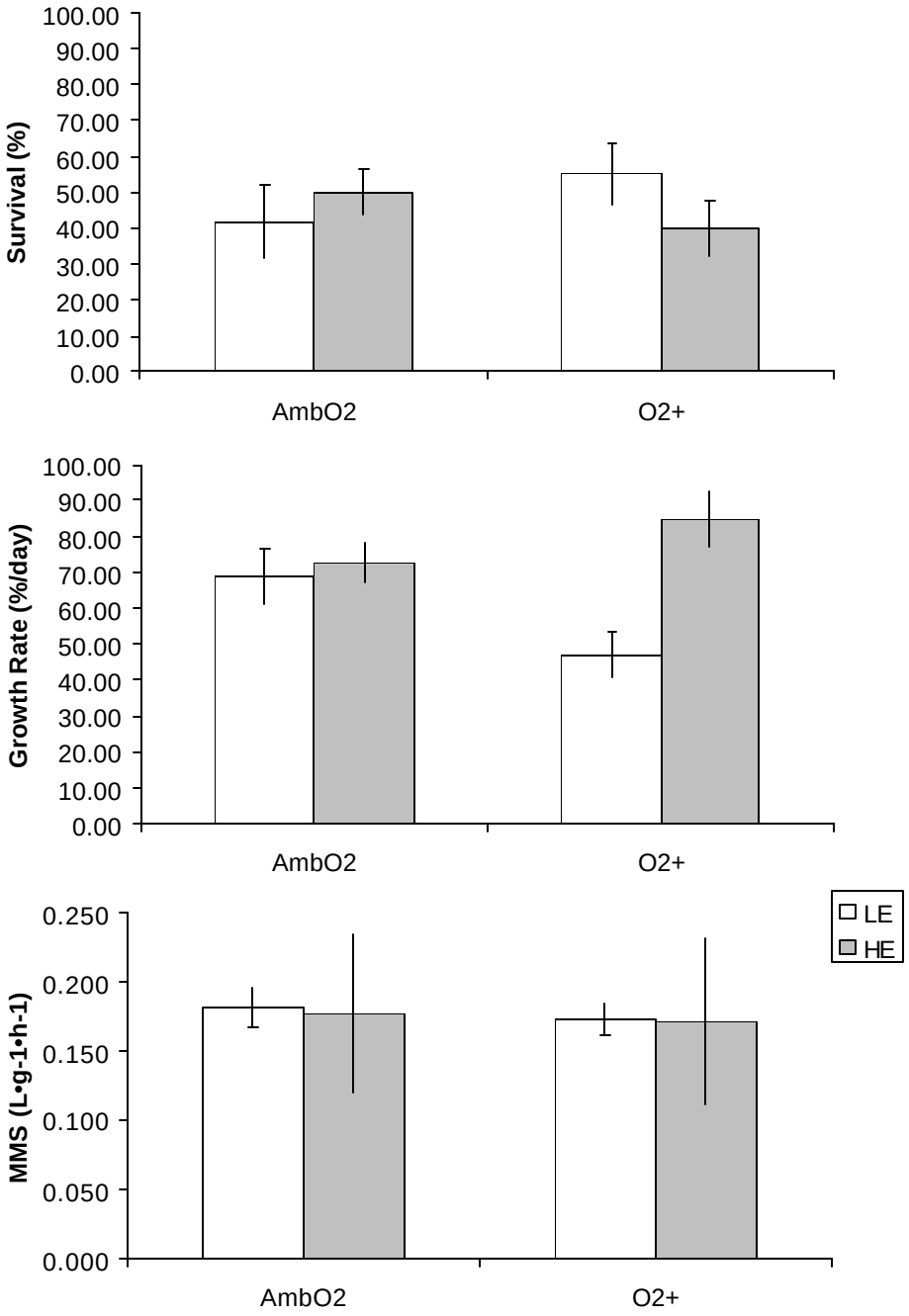

Figure 5.4. Survival (%), growth rate (%/day), and marginal metabolic scope (MMS; ($L \cdot g^{-1} \cdot h^{-1}$) of red drum consuming either a low-energy basal diet (LEb) or a high-energy basal diet (HEb) and exposed to an environmental regime supplemented with oxygen (O_2+) or one with ambient oxygen levels ($AmbO_2$) during a 41-day feed-oxygen experiment. Values are presented as mean ± SE for all fish from six replicate cages per treatment. Significance established via analysis of variance at $P < 0.05$.

Modeling

Ecophys.Fish simulations were not performed for the dietary supplementation study as the model currently does not have explicit inputs to accommodate dietary additives. While the influence of these additives could be rendered in the form of indirect effects on parameters that Ecophys.Fish does have (i.e. changes in the apparent digestibility of feed or in the physiological response of the fish), I felt that simulations of the feed-oxygen study would be more informative and, thus, a better investment of my time.

Ecophys.Fish was able to acceptably simulate observed performance of the HE AmbO2 treatment using "nominal" model input parameters with a MMSO value of 0.249 and a Winberg-adjustment of 1.2 (Table 5.3; Figure 5.5). Using the "nominal" input parameters, however, Ecophys.Fish was not able to reasonably simulate growth and metabolism simultaneously for those fish receiving the LE diet or the oxygen supplementation treatment. To obtain an adequate match for the LE dietary treatment, optimization of "nominal" model parameters was required. The value of input gross dietary energy for the LE diet (3.3 kJ DE/g on a fresh-weight basis) was altered to mimic the composition of the HE diet (15.9 kJ DE/g on a dry-weight basis), because Ecophys.Fish was dramatically underestimating final weight of those fish consuming the LE diet. When using 0.245 as the value of MMSO and the Winberg-adjustment set to 1.2, simulated performance of the LE AmbO_2 treatment closely matched observed.

Similarly, the "nominal" input for observed DO under oxygen-supplementation (i.e. a null-effect of O_2 addition on DO levels within a cage) would result in identical

Table 5.3. Comparison of observed versus Ecophys.Fish-simulated growth and metabolic responses for red drum in a 41-day feed-oxygen experiment. Values of MMSO and Winberg-adjustment necessary to achieve adequate agreement between observed and simulated fish performance are provided. Simulations using Ecophys.Fish suggest DO during periods of oxygen-supplementation may have been ~ 2 mg/L higher than ambient levels. The term, "nominal diet" means Ecophys.Fish was executed with inputs "as observed"; whereas, "HE diet" means the model was run with Feed energy inputs set to emulate the high-energy diet.

Experimental Treatment		Growth Rate (%/day)	Routine metabolic rate ($mgO_2 \cdot g^{-1} \cdot h^{-1}$)	Marginal metabolic scope ($L \cdot g^{-1} \cdot h^{-1}$)	MMSO	Winberg-adjustment
LE $AmbO_2$	Observed	65.62	0.354	0.178	-	-
	Simulated - (nominal diet)	-1.98	0.337	0.178	0.078	2.0
	Simulated - (HE diet)	64.34	0.342	0.182	0.245	1.2
LE O_2+	Observed	45.83	0.277	0.181	-	-
	Simulated - (nominal diet)	7.93	0.358	0.190	0.169	1.1
	Simulated - (HE diet)	46.67	0.346	0.184	0.233	1.2
HE $AmbO_2$	Observed	72.16	0.349	0.180	-	-
	Simulated	71.68	0.365	0.181	0.249	1.2
HE O_2+	Observed	87.90	0.301	0.166	-	-
	Simulated	87.95	0.313	0.167	0.250	1.2

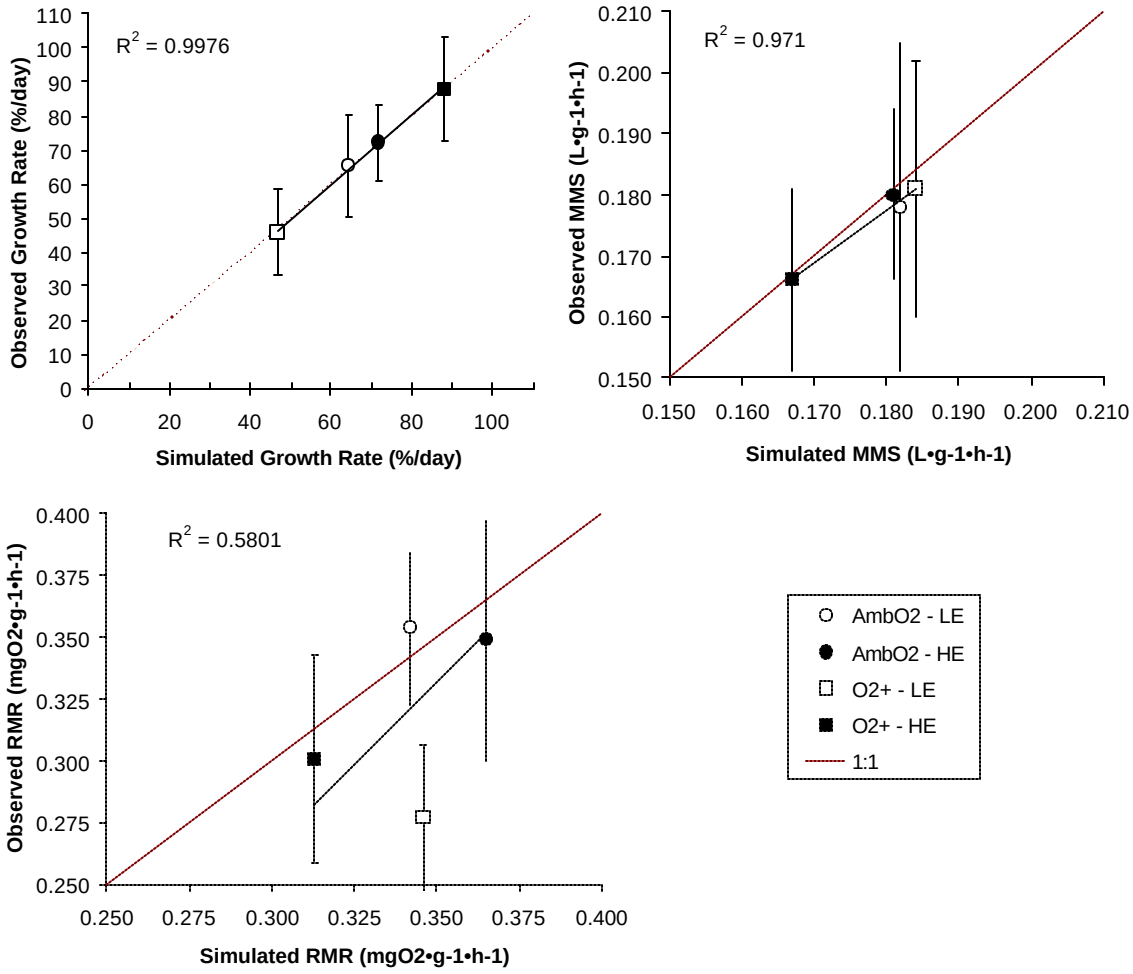

Figure 5.5. Comparison of observed versus Ecophys.Fish-simulated a.) growth rate (%/day; Mean +/- 95% Confidence Interval); b.) marginal metabolic scope (MMS; L g-1 h-1; Mean +/- 95% Confidence Interval); c.) routine metabolic rate (RMR; mgO_2 g-1 h-1; Mean +/- 95% Confidence Interval) for red drum in the feed-oxygen study. Note: simulated values are based on the *optimized* model in which dietary energy was estimated to be ~ 3.5 kcal DE/g and DO was estimated to be ~ 2 mg/L higher than recorded levels.

output from Ecophys.Fish. Here as well, however, it was impossible to achieve an adequate match between observed and simulated fish performance assuming a null-effect of O_2-supplementation. Furthermore, significant differences that are a function of oxygen-supplementation were detected in the data. One possible conclusion is that the actual levels of DO experienced by fish in O_2+ cages must have differed from those in the $AmbO_2$ cages, despite negligible differences in recorded values. Thus, nocturnal DO levels for the O_2+ treatments were manipulated until an accurate match between simulated and observed fish performance was obtained.

Following simulation procedures described by Clark (2003), Vega (2003), Neill et al. (2004), and Fontaine et al. (2007), ambient DO during periods of supplementation was increased step-wise, with a constant increment, using the following algorithm:

(1) Ecophys.Fish *simulated* performance ~ *observed* performance given:

(2) O_2+ = $AmbO_2$ + 0.0 mg/L;

(3) O_2+ = $AmbO_2$ + 0.5 mg/L;

(4) O_2+ = $AmbO_2$ + 1.0 mg/L;

(5) …. (increase by 0.5 mg/L at each iteration);

(6) terminate simulations when (1) is true.

At each iteration MMSO and Winberg-adjustment were simultaneously manipulated until observed and simulated RMR, MMS, and growth were deemed an adequate match. The algorithm was executed until no further improvement of fit was obtained, at which point the initial value of O_2+ that resulted in a match was accepted. In this manner, a

conservative estimate of the effect of O_2-supplementation on DO was achieved; it was (effective O_2+ = AmbO_2 + 2.0 mg/L).

Using the optimized diet and O_2 regime model inputs, simulated performance in the LE O_2+ treatment matched closely to that of observed with a MMSO value of 0.233 and a Winberg-adjustment of 1.2. With the same optimized O_2 regime, the model was able to very accurately simulate growth and metabolic performance of the HE O_2+ treatment using 0.250 as the value of MMSO and 1.2 as the Winberg-adjustment value. Thus, using a combination of nominal and carefully-optimized model input parameters, Ecophys.Fish simulations of fish performance closely matched those observed (Table 5.3; Figure 5.5).

DISCUSSION

In this study, the addition of the supplement GroBiotic®A to the experimental diets did not yield a net increase in survival, growth, or metabolic performance. Rather, survival was negatively impacted by the addition of the supplement to both low- and high-energy diet formulations; neither growth nor metabolic performance was differentially affected by the dietary supplement. A previous laboratory-based feeding trial using the same high-energy diets demonstrated a similar decrease in survival; however, that decrease occurred with a concordant increase in growth and decrease in MMS for those fish consuming the supplemented formulation (Fontaine unpublished data). In the latter case cannibalism may have contributed to the observed tendency for higher rates of mortality and subsequent increase in growth as fish were stocked at a higher density of ~ 20 individuals per 110-liter aquarium. Red drum are known to

achieve high rates of growth when consuming diets rich in red drum and other high-protein fish meal (Moon and Gatlin 1994; Whiteman and Gatlin 2005). In contrast, individuals in the current dietary-supplementation experiment were stocked at a much lower density of 4 individuals per cubic meter. Thus, cannibalism of up to three cage-mates—as opposed to nearly 5 times that amount in the laboratory study—would not have provided the same degree of high energy and well-balanced nutrition. This argument is further supported in that individuals consuming the high-energy ration grew significantly larger.

Previous laboratory experiments inducing environmental stressors—such as extremes of temperature and dissolved oxygen—indicate that those red drum consuming high-energy diets tend to perform better than their counterparts consuming a low-energy diet (Gaylord and Gatlin 1996; Gatlin 2002a; Gatlin 2002b; Grey 2003; Li et al. 2005; Fontaine et al. 2007). In this study, the lack of differences in growth and metabolic performance of fish consuming the supplemented diet across dietary energy levels suggests a mitigating effect of this supplement in situations of low digestible energy and optimum—or at least adequate—environment. Indeed, while salinity was slightly higher than the seasonal-norm, the overall 30-day environmental regime experienced by the red drum (Figure 5.1) in this study was within regional and historical expectations (Gunter 1945; Vega 2003) as well as the species' tolerances (Gunter 1979; Peters 1987; Matlock 1990; Neill 1990; Procarione and King 1993).

Presumably, an effect of GroBiotic®A to "level the playing field" would especially benefit individuals consuming the low-energy diet, had the environmental

regime reached stressful—and limiting—extremes. A beneficial effect of GroBiotic®A on growth of red drum receiving the high-energy diet—in "optimal", "sub-optimal", or non-limiting environments—remains unresolved. The supplement, however, is considered to impart non-growth related benefits—such as enhanced disease resistance and improved digestibility of feeds—in hybrid striped bass (Li and Gatlin 2003; Li et al. 2004; Li and Gatlin 2006); such performance measures were not addressed in this study. However, my finding is not inconsistent with laboratory studies involving red drum consuming prebiotic-supplemented feeds (Li et al. 2005).

In the feed-oxygen study described here, effects of feed energy on growth rate were consistent with results from the feed study, in that fish receiving the ration high in dietary energy grew significantly larger than their counterparts receiving the low-energy diet. A differential effect of dietary energy in the presence of oxygen-supplementation was detected; fish consuming the high-energy diet under oxygen-rich conditions grew nearly twice as large as their counterparts consuming the low-energy diet; for fish fed the low-energy diet, oxygen supplementation tended to reduce growth. Contrary to what was expected, however, oxygen supplementation did not have measurable effects on metabolic capacity of caged juvenile red drum in the present pond study. Oxygen-supplementation did, on the other hand, significantly reduce the RMR of fish across dietary treatments.

Growth—and to some degree MMS—is better able to capture long-term fish performance compared to RMR which tends to be more sensitive to short-term environmental and physiological fluctuations (Fry 1947; Fry 1971; Neill and Bryan

1991). Thus, in the oxygen-supplementation study, both long- and short-term metrics failed to demonstrate an enhancement of performance resulting from higher nocturnal levels of dissolved oxygen. To better understand these seemingly contradictory results Ecophys.Fish was employed to simulate fish performance given the conditions experienced by the fish in this study. Initial input parameters and assumptions for simulations (i.e. "nominal" simulations) were established as described above; nutritional attributes of the experimental diets were "as fed" and environmental values were as recorded adjacent to the submerged cages. Then, by making systematic and educated assumptions, I was able to manipulate inputs in order to explore potential causal mechanisms that may have influenced observed results.

Despite a consistent metabolic response between observed and nominally-simulated fish performance, growth rate and MMSO differed drastically for the two dietary energy levels (Table 5.3). Simulations using the nominal, non-supplemented LE diet suggest that those fish in the ambient O_2 ($AmbO_2$) regime should have been ~ 87 % smaller than was actually observed. By assuming that the fish in the LE treatment were consuming a diet with a different energetic density than presented, however, I was able to greatly improve the match to observed fish performance; the fish grew *as if* they were consuming a diet similar in composition and digestibility to the HE diet. While the model cannot indicate the source from which this "extra" dietary energy was available, it is informative when combined with data from zooplankton samples obtained regularly throughout the experiment. These data indicate an abundance of planktonic organisms— such as adult and naupliar stages of copepods—in the ponds during the

study period (TPWD unpublished data). These organisms are known sources of nutritionally-rich forage for juvenile red drum and could easily pass through the relatively large diameter mesh of the cages (Strumer 1990; Treece 1990; Treece and Wohlschlag 1990). Given the relatively small initial size of red drum at stocking, it is further reasonable to assume that the caged fish were consuming—or at least able to consume—these planktonic organisms.

I was logistically unable to obtain direct, in-cage measurements of any DO increase attributable to the oxygen supplementation efforts. This confounded my ability to interpret and evaluate fish performance results to the extent I had desired. Here as well, Ecophys.Fish, provided the ideal platform from which I was able to speculate on the environmental conditions actually experienced by the caged fish—in this case, the actual concentration of DO within the cages receiving the O_2+ treatment during periods of oxygen-supplementation. Simulations generated using this methodology suggest *it is reasonable* that the addition of oxygen-gas to O_2+ treatment cages increased DO during this period by ~ 2 mg/L (Table 5.3 and Figure 5.4). Interestingly, both simulated and observed results suggest that the DO regime in this study was not hypoxic enough to act as a limiting factor on red drum growth or performance. Presumably, if the nocturnal DO had been acting as a limiting factor, a significant effect of O_2 supplementation would have been observed both in the ponds and, subsequently, in Ecophys.Fish simulations.

To fully interpret the simulation results, it is critical to note the values of MMSO and Winberg-adjustment that were necessary to achieve adequate goodness-of-fit between observed and simulated performance (Table 5.3 and Figure 5.4). Recall that

under Ecophys.Fish, the Winberg-adjustment is meant to represent the multiple of standard metabolism used for routine metabolism (Neill et al. 2004); whereas, the parameter MMSO is used by Ecophys.Fish to quantify the inherent metabolic efficiency of the fish-environment system once all the other input variables have been considered (Neill and Bryan 1991; Neill et al. 2004; Fontaine et al. 2007). The Winberg-adjustments necessary to achieve adequate fit in all the optimal simulations in this study were identical, while the values of MMSO were highest for those fish that grew the greatest. These simulation results reinforce the idea that environment was consistent—and non-limiting—across treatments.

The values of Winberg-adjustment and MMSO from this study are congruent with those previously obtained in similar studies (Neill et al. 2004; Clark 2003; Vega 2003; Fontaine et al. 2007). It is noteworthy that the red drum utilized in all these studies were obtained from the same Texas Parks and Wildlife Department's hatchery facilities. Current genetic evidence of red drum in the Gulf of Mexico suggests greater genetic divergence with increasing spatial distance; however, a certain degree of genetic inter-mixing and overlap is thought to occur (Gold 1999; Gold and Turner 2002). Further research examining potential differences in red drum performance at other scales—regional, ecosystem, population—could be informative. These genetic differences may subsequently influence growth and metabolic performance characteristics of red drum given different environmental and nutritional circumstances.

CHAPTER VI

CONCLUSIONS

The purpose of this chapter is to discuss methodological considerations in the use of Ecophys.Fish for planning and interpreting ecophysiological experiments and to present a brief synopsis of my research findings. The following should be of particular interest to those who would adopt Ecophys.Fish to facilitate their own investigations of fish ecophysiology and especially to those involved in aquaculture and management of red drum.

GENERAL CONCLUSIONS

1) Growth of red drum exposed to higher temperatures (~29°C) can be limited by available food energy; whereas, growth of fish exposed to lower temperatures (~18°C) can be limited by their metabolic capacity to exploit available food energy.
2) Growth rate and marginal metabolic scope (MMS) increased with temperature, but only growth rate increased with dietary energy at higher temperatures.
3) Greater differences between the metabolic response of red drum consuming the two experimental diets at lower temperature versus higher temperature provide corroborative evidence that fish in the cooler regime did not have the metabolic capacity to process the additional nutrients and energy available in the HE diet.
4) Low dissolved oxygen (DO) was limiting to growth and metabolism at the higher temperature; under this combination of environmental conditions, limiting effects

of low feed energy on overall performance were amplified compared to a regime with low temperature and low DO.

5) Within the cyclical environment, growth rate remained an energy-dependent response; however, the specific cyclic diel environment imposed here did not promote the anticipated enhancement of growth.
6) The feed efficiency, hepatosomatic index, intraperitoneal fat ratio, and whole-body fat of red drum fed a low-energy (LE) diet were significantly lower than those of fish fed a high-energy (HE) diet, indicating relative energy malnutrition in the LE group.
7) Ecophys.Fish simulations were consistent with results of laboratory trials in that performance of red drum is enhanced at warmer temperatures, especially for those fish consuming an energetically-dense diet.
8) In a hatchery pond setting, the dietary supplement GroBiotic®A tended to decrease survival of caged juvenile red drum; however, the lack of differences in growth and metabolic performance for fish consuming the supplemented diet across dietary energy levels suggests a mitigating effect of this supplement in situations of low digestible energy and "adequate" environment.
9) In a hatchery pond setting, red drum consuming the HE diet under the oxygen-supplementation treatment grew significantly faster than their counterparts not receiving additional oxygen.
10) Simulation modeling of the hatchery pond trials using Ecophys.Fish suggested a) that fish receiving the LE diet were consuming a diet higher in caloric density

than intended and b) that observed nocturnal levels of DO did not limit either growth or metabolic capacity.

11) The Winberg-adjustment tended to be positively correlated with MMSO.
12) Red drum obtain greater metabolic capacity when exposed to a near optimal temperature and their ability to transform that capacity into growth is maximized only when they are provided a nutritious, high-energy diet.

METHODOLOGICAL CONSIDERATIONS

Initially, I utilized Ecophys.Fish to help develop research questions and hypotheses. Ecophys.Fish allowed for the exploration and refinement of various environmental and nutritional treatment scenarios with minimal expense as measured in time, funding, and potential fish mortality. Once experimentation began, Ecophys.Fish was used to ensure that feeding regimes were presented according to the experimental protocol. Finally, Ecophys.Fish was used to assist with data interpretation by providing simulated results for comparison with observed results. If discrepancies existed between actual and simulated outcomes, the model was used to explore *possible* reasons as to why results were at variance from those expected under the nominal Ecophys.Fish model. When utilizing Ecophys.Fish in such a manner, it is very important to indicate and emphasize that these speculations cannot always be confirmed or investigated further in every instance. Nevertheless, such utilization of Ecophys.Fish provides a useful and informative platform with which educated and systematic explanations may be developed.

Another important and distinguishing aspect of this approach is that analyses involving Ecophys.Fish are separate and distinct from traditional statistical analyses. Indeed, each experiment should be designed, executed, and statistically evaluated with standard scientific rigor. The strength of Ecophys.Fish is that it allows for the exploration of the data in ways different from those permitted by traditional statistics. This is especially true for responses that are in transient state.

Ecophys.Fish serves as an interface between observed results and future research to further the resolution and understanding of underlying processes.

RESEARCH SYNOPSIS

The underlying goal of this research was to improve understanding of the influence of abiotic effects on juvenile red drum performance. Specifically, I hoped to elucidate some of the complex interactions of environment and nutrition on survival, growth, and metabolism of this euryhaline sciaenid. The intent was to improve the knowledge-base such that hatchery managers might more effectively rear red drum and fishery biologists better manage wild red drum in the context of environmental variation. In the context of stock enhancement, it may seem inappropriate to group hatchery-raised and wild fish due to apparent differences in their early life history. Fundamentally, however, the ecophysiology of these two groups must be convergent, once they start to share common ecological experiences in estuarine and pelagic ecosystems. The incorporation of Ecophys.Fish into the methodology exploits this commonality and is an integral component to the multifaceted strategy I used to address the three broad objectives of this research.

As mentioned throughout this text, a direct statistical comparison across all experiments was not appropriate for various reasons. Appendix B, however, provides the reader with a "quick-reference" type tabular display of observed growth rate and the two most critical Ecophys.Fish parameters—MMSO and the Winberg-adjustment—and will be useful for subsequent research involving Ecophys.Fish. This table, in conjunction with the broader findings described here, provide strong support for the "energy/metabolism-tradeoff hypothesis." This hypothesis suggests that there is an enabling effect of elevated temperature on metabolism such that growth is maximized if food is ample, nutritionally adequate, and energy dense (and that no other environmental factor is limiting). Indeed, consistent throughout this study, juvenile red drum—although adaptable to a broad range of abiotic conditions—prospered under "warmer" water conditions (here, ~25°C to ~29°C), with DO at or near air-saturation, while consuming a nutritious and energetically-dense (15.9 kJ/g) diet.

My experiments did not demonstrate any "out-performance" by red drum subjected to cyclic regimes of temperature and dissolved-oxygen concentration in the laboratory, nor any benefit of oxygen-supplementation in the pond trials with caged fish. Application of Ecophys.Fish indicated that lack of expected effects may have been caused, not by fundamental faults in the model, but by faults in experimental design. This is to say, the imposed environmental cycles may have lacked the characteristics that would cause out-performance, and the values of DO and feed energy recorded in the pond-cage experiment may have been different from those actually experienced by the fish.

The values of Winberg-adjustment and MMSO from this study are congruent with those previously obtained in similar studies (Clark 2003; Vega 2003; Neill et al. 2004; Fontaine et al. 2007).

It is perhaps important to note that the red drum utilized in all these studies were obtained from the same three Texas Parks and Wildlife Department's hatchery facilities. Current genetic evidence of red drum in the Gulf of Mexico suggests greater genetic divergence with increasing spatial distance; however, a certain degree of genetic inter-mixing and overlap is thought to occur. Further research examining potential differences in red drum performance at other scales—regional, ecosystem, population—could be informative. These genetic differences may subsequently influence growth and metabolic performance characteristics of red drum given different environmental and nutritional circumstances.

In hatchery and aquacultural settings, the adaptable nature of the red drum allows managers to mitigate losses due to ephemeral periods of poor environment and nutrition. Conversely, managers may choose to manipulate environmental and nutritional regimes in order to fully exploit the robust energy-processing and metabolic capabilities of red drum. The latter practice should only be attempted cautiously and while incorporating Ecophys.Fish to aid in wise planning and proper interpretation of results and outcomes.

REFERENCES

AOAC. (Association of Official Analytical Chemists) 1990. Pages 71-74 *in* K. Helrich editor. Official methods of analysis of the Association of Official Analytical Chemists. 15th edition. Arlington, Virginia.

Arnold, C. R. 1988. Controlled year-round spawning of red drum Sciaenops ocellatus in captivity. Contributions in Marine Science Supplement to volume 30:65-70.

Azevedo, P. A., C. Y. Cho, S. Leeson, and D. P. Bureau. 1998. Effects of feeding level and water temperature on growth, nutrient and energy utilization and waste outputs of rainbow trout (*Oncorhynchus mykiss*). Aquatic Living Resources 11:227-238.

Baker, D. H. 1986. Problems and pitfalls in animal experiments designed to establish dietary requirements for the essential nutrients. Journal of Nutrition 116:2339-2349.

Barton, B. A., and G. K. Iwama. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Review of Fish Diseases 1:3-26.

Blankenship H. L., and K. M. Leber. 1995. A responsible approach to marine stock enhancement. American Fisheries Society Symposium 15:167-175.

Blaxter, J. H. S. 2000. The enhancement of marine fish stocks. Advances in Marine Biology 38:54.

Boothby, R. N. and J. W. Avault Jr. 1971. Food habits, length-weight relationship, and condition factor of the red drum (*Sciaenops ocellatus*) in southeastern Louisiana. Transactions of the American Fisheries Society 2:290-295.

Borda, E., D. Martinez-Puig, and X. Cordoba. 2003. A balanced nucleotide supply makes sense. Feed Mix 11:24-26.

Brett, J. R. 1971. Energetic responses of salmon to temperature: a study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhychus nerka*). American Zoologist 11:99-113.

Brett, J. R. 1979. Environmental factors and growth. Pages 599-675 *in* W. S. Hoar and D. J. Randall, editors. Fish physiology No. VIII. Academic Press, Inc., New York.

Brett, J. R., and T. D. D. Groves. 1979. Physiological energetics. Pages 279-352 *in* W.S. Hoar, D. J. Randall, and J. R. Brett, editors. Fish physiology, Volume 8. Academic Press, New York.

Buentello, J. A., W. H. Neill, and D. M. Gatlin III. 2000. Effects of water temperature and dissolved oxygen on daily feed consumption, feed utilization and growth of channel catfish (*Ictalurus punctatus*). Aquaculture 82:339-352.

Burrells, C., P. D. William, and P. F. Forno. 2001a. Dietary nucleotides: a novel supplement in fish feeds 1. Effects on resistance to diseases in salmonids. Aquaculture 199:159-169.

Burrells, C., P. D. William, P. J. Southage, and S. L. Wadsworth. 2001b. Dietary nucleotides: a novel supplement in fish feeds 2. Effects on vaccination, salt water

transfer, growth rate and physiology of Atlantic salmon. Aquaculture 199:171-184.

Chamberlain, G. C., R. J. Miget, and M. G. Haby. 1990. Red drum aquaculture. Proceedings of a symposium on the culture of red drum and other warm water fishes. Texas Sea Grant College Program, TAMU-SG-90-603, Galveston, Texas.

Chang, Y. J., M. H. Jeong, B. H. Min, W. H. Neill, and L. P. Fontaine. 2005. Effects of photoperiod, temperature, and fish size on oxygen consumption in the Black Porgy *Acanthopagrus schlegeli*. Journal of Fisheries Science and Technology 8:142-150.

Clark, K. W. 2003. Marginal metabolic scope and growth of hatchery-produced, juvenile red drum by progeny group. Master's thesis. Texas A&M University, College Station, Texas.

Colura, R. L., B. W. Bumguardner, A. Henderson-Arzapalo, and J. D. Gray. 1990. Culture of red drum fingerlings. Texas Parks and Wildlife Department, Coastal Fish Division, Management Data Series 22, Austin, Texas.

Colura, R. L., B. T. Hysmith, and R. E. Stevens. 1976. Fingerling production of striped bass (*Morone saxatilis)* spotted seatrout (*Cynoscion nebulosus*), and red drum (*Sciaenops ocellatus*) in saltwater ponds. Proceedings of the World Mariculture Society 7:79-92.

Craig, S. R., D. S. MacKenzie, G. Jones, and D. M. Gatlin III. 2000. Seasonal changes in the reproductive condition and body composition of free-ranging red drum, *Sciaenops ocellatus*. Aquaculture 190:89-102.

Craig, S. R., W. H. Neill, and D. M. Gatlin III. 1995. Effects of dietary lipid and environmental salinity on growth, body composition, and cold tolerance of juvenile red drum (*Sciaenops ocellatus*). Fish Physiology and Biochemistry 14:49-61.

Craig, S. R., B. S. Washburn, and D. M. Gatlin III. 1999. Effects of dietary lipids on body composition and liver function in juvenile red drum, *Sciaenops ocellatus*. Fish Physiology and Biochemistry 21:249-255.

Davis, K. B., and B. R. Griffin. 2004. Physiological responses of hybrid striped bass under sedation by several anesthetics. Aquaculture 233:531-548.

Diamant, A. 1998. Red drum *Sciaenops ocellatus* (Sciaenidae), a recent introduction to Mediterranean mariculture, is susceptible to *Myxidium leei* (Myxoporea). Aquaculture 162:33-39.

Dickerson, B. R., and G. L. Vinyard. 1999. Effects of high chronic temperatures and diel temperature cycles on the survival and growth of Lahontan cutthroat trout. Transactions of the American Fisheries Society 128:516-521.

Fontaine, L. P. 2002. Fish-performance ecoassay of urbanizing streams in the San Antonio River basin, Texas. Master's thesis. Texas A&M University, College Station, Texas.

Fontaine, L. P., K. W. Whiteman, P. Li, G. S. Burr, K. A. Webb, J. Goff, D. M. Gatlin III, W. H. Neill, K. B. Davis, and R. R. Vega. 2007. Effects of temperature and feed energy on performance of juvenile red drum. Transactions of the American Fisheries Society 136:1193-1205.

Forsberg, J. A., and W. H, Neill. 1997. Saline groundwater as an aquaculture medium: physiological studies on the red drum, *Sciaenops ocellatus*. Environmental Biology of Fishes 49:119-128.

Fox, H. M., and B. G. Simmonds. 1932. Metabolic rate and habitat. Nature 130:277-278.

Fry, F. E. J. 1947. Effects of the environment on animal activity. University of Toronto Studies, Biological Series 55:1-62.

Fry, F. E. J. 1971. The effect of environmental factors on the physiology of fish. Pages 1-98 *in* W. S. Hoar and D. J. Randall, editors, Fish physiology: environmental relations and behavior. Academic Press, New York.

Fushimi, H. 2001. Production of juvenile marine finfish for stock enhancement in Japan. Aquaculture 200:33-53.

Gatlin, D. M., III, W. E. Poe and R. P. Wilson. 1986. Protein and energy requirements of fingerling channel catfish for maintenance and maximum growth. Journal of Nutrition 116:2121-2131.

Gatlin, D. M., III. 2000. Red drum culture. Pages 736-741 *in* R. R. Stickney, editor. Encyclopedia of aquaculture. John Wiley & Sons, New York.

Gatlin, D. M., III. 2002a. Nutrition and fish health. Pages 671-702 *in* J. E. Halver and R. W. Hardy. editors. Fish nutrition. Academic Press, Boston, Massachusetts.

Gatlin, D. M., III. 2002b. Red drum *Sciaenops ocellatus*. Pages 147-158 *in* C. D. Webster and C. Lim. editors. Nutrient requirements and feeding of finfish for aquaculture. CABI Publishing, Wallingford, Oxon, United Kingdom.

Gaylord, T. G., and D. M. Gatlin III. 1996. Determination of digestibility coefficients of various feedstuffs for red drum (*Sciaenops ocellatus*). Aquaculture 139:303-314.

Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of size and temperature on metabolic rate. Science 293:2248-2251.

Gold, J. R. 1999. Stock structure and effective size of red drum (Sciaenops ocellatus) in the northern Gulf of Mexico and implications relative to stock enhancement and recruitment. Page 606 *in*: K. M. Leber, S. Kitada, H. L. Blankenship, and T. Svasand, editors. Stock enhancement and sea ranching. Oxford Press, Malden, Massachusetts.

Gold, J. R., and T. F. Turner. 2002. Population structure of red drum (*Sciaenops ocellatus*) in the northern Gulf of Mexico, as inferred from variation in nuclear-encoded microsatellites. Marine Biology 140:249-265.

Goodyear, C. P. 1989. Status of the red drum stocks of the Gulf of Mexico. Southeast Fisheries Center, Miami Laboratory, Coastal Resources Division, Contribution number CRD 88/89-14, Miami, Florida.

Grey, M. S. 2003. Feeding and feed-processing by red drum (*Sciaenops ocellatus*) fed natural and formulated diets. Master's thesis. Texas A&M University, College Station, Texas.

Gunter, G. 1945. Some characteristics of ocean waters and Laguna Madre. Texas Game Fish 3:7-9.

Gunter, M. P. 1979. Studies on the time course of acclimation to salinity changes in

juvenile spotted seatrout and red drum. Doctoral dissertation. University of Texas, Austin, Texas.

Hokanson, K. E. F., C. F. Kleiner, and T. W. Thorslund. 1977. Effects of constant temperature and diel temperature fluctuations on specific growth and mortality rates and yield of juvenile rainbow trout, *Salmo gairdneri*. Journal Fisheries Research Board of Canada 34:639-648.

Hong, W., and Q. Zhang. 2003. Review of captive bred species and fry production of marine fish in China. Aquaculture 227:305-318.

Hopkins, J. S., T. I. J. Smith, A. D. Stokes, and P. A. Sandifer. 1988. Winter survival of fingerling red drum (*Sciaenops ocellatus)* in South Carolina culture ponds. Contributions in Marine Science Supplement to Volume 30:5-10.

Hubbs, C. 1964. Effects of thermal fluctuations on the relative survival of greenthroat darter young from stenothermal and eurythermal waters. Ecology 45:376-379.

Jenkins, W. E., T. I. J. Smith and M. R. Denson. 2004. Stocking red drum: lessons learned. American Fisheries Society Symposium 44:45-56.

Kendal, R. L., I. Coolen, and K. N. Laland. 2004. The role of conformity in foraging when personal and social information conflict. Behavioral Ecology 15:269-277.

Kilic, M., E. Taskin, B. Ustundag, and A. D. Aygun. 2004. The evaluation of serum leptin level and other hormonal parameters in children with severe malnutrition. Clinical Biochemistry 37:382-387.

Kristiansen, T. S., A. Ferno, J. C. Holm, L. Privitera, S. Bakke, and J. E. Fosseidengen.

2004. Swimming behaviour as an indicator of low growth rate and impaired welfare in Atlantic halibut (*Hippoglossus hippoglossus* L.) reared at three stocking densities. Aquaculture 230:137-151.

Lakshmi, G. J., A. Venkataramiah, and G. Gunter. 1976. Effects of salinity and photoperiod on the burying behavior of brown shrimp *Penaeus aztecus* Ives. Aquaculture 8:327-336.

Lara-Flores, M., M. A. Olvera-Novoa, B. E. Guzmán-Méndez, and W. López-Madrid. 2002. Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquaculture 216:193-201.

Lee, C. S. 1997. Marine finfish hatchery technology in the USA - status and future. Hydrobiologia 358:45-54.

Lee, C. S., and A. C. Ostrowksi. 2001. Current status of marine finfish larviculture in the United States. Aquaculture 200:89-109.

Li, P., G. S. Burr, J. B. Goff, K. W. Whiteman, K. B. Davis, R. R. Vega, W. H. Neill, and D. M. Gatlin III. 2005. A preliminary study on the effects of dietary supplementation of brewers yeast and nucleotides, singularly or in combination, on juvenile red drum *Sciaenops ocellatus*. Aquaculture Research 36:1120-1127.

Li, P., and D. M. Gatlin III. 2003. Evaluation of brewers yeast (*Saccharomyces cerevisiae*) as a feed supplement for hybrid striped bass (*Morone chrysops* × *M. saxatilis*). Aquaculture 219:681-692.

Li, P., and D. M. Gatlin III. 2006. Nucleotide nutrition in fish: current knowledge and future application. Aquaculture 251:141-152.

Li, P., D. H. Lewis, and D. M. Gatlin III. 2004. Evaluation of dietary nucleotides as immunomodulator for hybrid striped bass (*Morone chrysops*×*M. saxatilis*). Fish and Shellfish Immunology 16:561-569.

Liao, I. C., M. S. Su, and E. M. Leaño. 2003. Status of research in stock enhancement and sea ranching. Reviews in Fish Biology and Fisheries 13:151-163.

Lorenzen, K. 2006. Population management in fisheries enhancement: gaining key information from release experiments through use of a size-dependent mortality model. Fisheries Research 80:19-27.

Lovell, T. 1998. Nutrition and feeding of fish. Kluwer Academic Publishers, Boston, Massachusetts.

Luebke, R. W., and R. K. Strawn. 1973. The growth, survival, and feeding behavior of redfish (*Sciaenops ocellatus*) in ponds receiving heated discharge water from a power plant. Proceedings of the World Mariculture Society 4:143-154.

Lyczkowski-Shultz, J., J. P. J. Steen, and B. H. Comyns. 1988. Early Life History of Red Drum (*Sciaenops ocellatus*) in the Northcentral Gulf of Mexico. Gulf Coast Research Laboratory, Ocean Springs, Mississippi.

Masuda, R., and K. Tsukamoto. 1998. Stock enhancement in Japan: review and perspective. Bulletin of Marine Science 62:337-358.

Matlock, G. C. 1990. The life history of red drum. Pages 1-21 *in* G. W. Chamberlin, R. J. Miget, and M. G. Haby, editors. Red Drum Aquaculture, Proceedings of a

Symposium on the Culture of Red Drum and Other Warm Water Fishes. Texas A&M University Sea Grant College Program, TAMU-SG-90-603, Galveston, Texas.

McGoogan, B. B., and D. M. Gatlin III. 1998. Metabolic requirements of red drum, *Sciaenops ocellatus*, for protein and energy based on weight gain and body composition. Journal of Nutrition 128:123-129.

McEachron, L. W., R. L. Colura, B. W. Bumguardner and R. Ward. 1998. Survival of stocked red drum in Texas. Bulletin of Marine Science 62:359-368.

McEachron, L. W. and K. Daniels. 1995. Red drum in Texas: a success story in partnership and commitment. Fisheries 20:6-8.

McEachron, L. W., C. E. McCarty, and R. R. Vega. 1993. Successful enhancement of the Texas red drum (*Sciaenops ocellatus*) population. Pages 53-56 *in* Interactions between cultured species and naturally occurring species in the environment: proceedings of the Twenty-Second U.S.-Japan Aquaculture Panel Symposium, Homer, Alaska.

McEachron, L. W., C. E. McCarty, and R. R. Vega. 1995. Beneficial uses of marine fish hatcheries: enhancement of red drum in Texas coastal waters. American Fisheries Society Symposium 15:161-166.

McLaren, I. A. 1963. Effects of temperature on growth of zooplankton and the adaptive value of vertical migration. Journal Fisheries Research Board of Canada 20:685-727.

Milliken, G. A., and D. E. Johnson. 1992. Analysis of messy data. Chapman & Hall,

New York.

Moon, H. Y. L. and D. M. Gatlin III. 1994. Effects of dietary animal proteins on growth and body composition of red drum (*Sciaenops ocellatus*). Aquaculture 120:327-340.

Munro, J. L., and J. D. Bell. 1997. Enhancement of marine fisheries resources. Reviews in Fisheries Sciences 5:185-222.

Mustafa, S. 2003. Stock enhancement and sea ranching: objectives and potential. Reviews in Fish Biology and Fisheries 13:141-149.

Mustafa, S., S. Saad, and R. A. Rahman. 2003. Species studies in sea ranching: an overview and economic perspectives. Reviews in Fish Biology and Fisheries 13:165-175.

National Instruments, Inc., 2000. LabView Software 5.1.1.

NMFS. (National Marine Fisheries Service) 1999. Our living oceans. Report on the status of U.S. living marine resources. United States Department of Commerce, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, NOAA Technical Memorandum NMFS-F/SPO-41, Silver Spring, Maryland.

NMFS. (National Marine Fisheries Service) 2004. Annual report to Congress on the status of U.S. fisheries - 2003. United States Department of Commerce, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Silver Spring, Maryland.

Neal, R. A. 1975. Shrimp culture research in controlled environments, Fish-Farming

Conference and Annual Convention of Catfish Farmers of Texas, College Station, Texas.

Neill, W. H. 1990. Environmental requirements of red drum. Pages 105-108 *in* G. W. Chamberlain, R. J. Miget, and M. G. Haby, editors, Red drum aquaculture. Texas A&M Sea Grant College Program, TAMU-SG-90-603, Galveston, Texas.

Neill, W. H., T. S. Brandes, B. J. Burke, S. R. Craig, L. V. DiMichele, K A. Duchon, L. P. Fontaine, D. M. Gatlin III, C. Hutchings, R. E. Edwards, J. M. Miller, B. J. Ponwith, C. J. Stahl, J. R. Tomasso, and R. R. Vega. 2004. Ecophys.fish: a simulation model of fish growth in time-varying environmental regimes. Reviews in Fisheries Sciences 12:233-288.

Neill, W. H., and J. D. Bryan. 1991. Responses of fish to temperature and oxygen, and response integration through metabolic scope. Pages 30-57 *in* D. E. Brune, and J. R. Tomasso, editors. Aquaculture and water quality (advances in world aquaculture, Volume 3). The World Aquaculture Society, Baton Rouge, Louisiana.

NRC (National Research Council). 1993. Page 114 *in* Nutrient Requirements of Fish. National Academy Press, Washington, DC.

Ortuño, J., A. Cuesta, A. Rodríguez, M. Angeles Esteban, and J. Meseguer. 2002. Oral administration of yeast, *Saccharomyces cerevisiae*, enhances the cellular innate immune response of gilthead seabream (*Sparus aurata* L.). Veterinary Immunology and Immunopathology 85:41-50.

Perez-Dominguez, R. 2004. Effects of nursery-environment conditions on habitat use,

growth, survival, and endocrine physiology during larval settlement in the red drum (*Sciaenops ocellatus*). Doctoral dissertation, University of Texas, Austin, Texas.

Perez-Dominguez, R., and G. J. Holt. 2002. Effects of nursery environmental cycles on larval red drum (*Sciaenops ocellatus*) growth and survival. Texas Water Resources Institute Special Report 02-02, College Station, Texas.

Pérez-Domínguez, R., and G. J. Holt. 2006. Interrenal and thyroid development in red drum (*Sciaenops ocellatus*): effects of nursery environment on larval growth and cortisol concentration during settlement. General and Comparative Endocrinology 146:108-118.

Peters, K. M., and R. J. McMichael Jr. 1987. Early life history of the red drum, *Sciaenops ocellatus* (Pisces: Sciaenidae), in Tampa Bay, Florida. Estuaries 10:92-107.

Phares, P. L. 1980. Temperature associated growth of white shrimp in Louisiana. United States Department of Commerce, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, NOAA Technical Memorandum NMFS-SEFC-56, Silver Spring, Maryland.

Plaia, W. C. 1987. A computerized environmental monitoring and control system for use in aquaculture. Aquacultural Engineering 6:27-37.

Powers, E. 1932. The relation of respiration of fishes to environment. Ecological Monographs 2:385-473.

Procarione, L. S., and T. L. King. 1993. Upper and lower temperature tolerance limits for juvenile red drum from Texas and South Carolina. Journal of Aquatic Animal Health 5:208-212.

Robinson, E. H. 1988. Nutritional requirements of red drum: a review. Contributions in Marine Science Supplement to Volume 30:11-20.

Rutledge, W. P. 1989. The Texas marine hatchery program - it works! California Cooperative Oceanic Fisheries Investigations Progress Report 30:49-52.

Sakai, M. 1999. Current research status of fish immunostimulants. Aquaculture 172:63-92.

Scharf, F. S. 2000. Patterns in abundance, growth, and mortality of juvenile red drum across estuaries on the Texas coast with implications for recruitment and stock enhancement. Transactions of the American Fisheries Society 129:1207-1222.

Serafy, J. E., J. S. Ault, T. R. Capo, and D. R. Schultz. 1999. Red drum, *Sciaenops ocellatus* L., stock enhancement in Biscayne Bay, FL, USA: assessment of releasing unmarked early juveniles. Aquaculture Research 30:737-750.

Serrano, J. A., G. R. Nematipour, and D. M. Gatlin III. 1992. Dietary protein requirement of the red drum (*Sciaenops ocellatus*) and relative use of dietary carbohydrate and lipid. Aquaculture 101:283-291.

Simmons, E. G., and J. P. Breuer. 1962. A study of redfish, *Sciaenops ocellata* Linnaeus, and black drum, *Pogonias cromis* Linnaeus. Publications of the Institute of Marine Science, University of Texas 8:184-211.

Soliman, A. T., M. M. El-Zalabany, M. Salama, and B. M. Ansari. 2000. Serum leptin concentrations during severe protein-energy malnutrition: correlation with growth parameters and endocrine function. Metabolism 49:819-825.

Springer, T. A., and W. H. Neill. 1988. Automated determination of critical oxygen concentration for routinely active fish. Environmental Biology of Fishes 23:233-240.

Stunz, G. W., T. J. Minello, and P. S. Levin. 2002. Growth of newly settled red drum *Sciaenops ocellatus* in different estuarine habitat types. Marine Ecology Progress Series 238:227-236.

Sturdevant, M. 2005. Hooked on fishing. Corpus Christi Caller Times. Corpus Christi, Texas.

Sturmer, L. N. 1990. Zooplankton composition and dynamics in fingerling red drum rearing ponds. Pages 66 -70 *in* G. W. Chamberlin, R. J. Miget, and M. G. Haby, editors. Red drum aquaculture. Texas A&M University Sea Grant College Program, Galveston, Texas.

Thacker, S., and W. L. Griffin. 1994. Indoor intensive red drum aquaculture: a stochastic sensitivity analysis. Journal of the World Aquaculture Society 25:86-100.

Tomasso, J. R. 1996. Environmental requirements and noninfectious diseases. Pages 253-270 *in* R. M. Harrell, editor. Developments in aquaculture and fisheries sciences. Elsevier, Houston, Texas.

Tidwell, J. H., and G. L. Allen. 2001. Fish as food: aquaculture's contribution. World

Aquaculture 11:958-963.

Tringali, M. D., and T. M. Bert. 1998. Risk to genetic effective population size should be an important consideration in fish stock-enhancement programs. Bulletin of Marine Science 62:641-659.

Treece, G. D. 1990. Raising food organisms for intensive larval culture: III. artemia. Pages 71 -71 *in* G. E. Chamberlin, R. J. Miget, M. G, Haby, editors. Red drum aquaculture. Texas A&M University Sea Grant College Program, Galveston, Texas.

Treece, G. D., and N. Wohlschlag. 1990. Raising food organisms for intensive larval culture: I. algae. Pages 57 – 65 *in* G. E. Chamberlin, R. J. Miget, and M. G, Haby, editors. Red drum aquaculture. Texas A&M University Sea Grant College Program, Galveston, Texas.

Turner, T. F., J. P. Wares, and J. R. Gold. 2002. Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependent marine fish (*Sciaenops ocellatus*). Genetics 162:1329-1339.

Van Weerd, J. H., and J. Komen. 1998. The effects of chronic stress on growth in fish: a critical appraisal. Comparative Biochemistry and Physiology 120A:107-112.

Vargas-Albores, F., P. Hinojosa-Baltazar, G. Portillo-Clark, and F. Magellon-Barajas. 1998. Influence of temperature and salinity on the yellowleg shrimp, *Penaeus claiforniensis* Holmes, prophenoloxidase system. Aquaculture Research 29:549-553.

Vega, R. R. 2003. Ecophysiological performance of hatchery-produced red drum (*Sciaenops ocellatus*) in culture ponds and in Texas coastal waters. Doctoral dissertation. Texas A&M University, College Station, Texas.

Vega, R. R., D. Abrego, R. J. Gamez, and A. Garza. 2007. Perspectives on Marine Stock Enhancement in Texas. Aquaculture 2007: Science for Sustainable Aquaculture. San Antonio, Texas.

Vega, R. R., C. Chavez, C. J. Stolte and D. Abrego. 2003. Marine fish distribution report, 1991-99. Texas Parks and Wildlife Department, Coastal Fish Division, Management Data Series No. 212, Austin, Texas.

Vega, R. R., C. E. McCarty, and W. P. Rutledge. 1995. The marine drums. Pages 319-326 *in* C. E. Nash and A. J. Novotny, editors. Production of aquatic animals (fishes). World animal science: production-system approach. Elsevier Science Publishers, Amsterdam.

Villarreal, H., P. Hinojosa, and J. Naranjo. 1994. Effect of temperature and salinity on the oxygen consumption of laboratory produced *Penaeus vannamei* postlarvae. Comparative Biochemistry and Physiology 108A:331-336.

Webb, K. A. and D. M. Gatlin III. 2003. Effects of dietary protein level and form on production characteristics and ammonia excretion of red drum *Sciaenops ocellatus*. Aquaculture 225:17-26.

Wedemeyer, G. A., B. A. Barton, and D. J. McLeay. 1990. Stress and acclimation. Pages 451-489 *in* C. B. Schreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

Weisepape, L. M., D. V. Aldrich, and R. K. Strawn. 1972. Effects of temperature and salinity on thermal death in postlarval brown shrimp, *Penaeus aztecus*. Physiological Zoology 45:22-33.

Wetherbee, B. M. and S. H. Gruber. 1993. Use of acid-insoluble ash as a maker in absorption efficiency studies with the lemon shark. Progressive Fish-Culturist 55:270-274.

Whiteman, K. W., and D. M. Gatlin III. 2005. Evaluation of fisheries by-catch and by-product meals in diets for red drum *Sciaenops ocellatus* L. Aquaculture Research 36:1572-1580.

Winberg, G. G. 1960. Rate of metabolism and food requirements of fishes. Fisheries Research Board of Canada, Translation Series 194, Ontario, Canada.

Wurts, W.A., and R. R. Stickney. 1989. Responses of red drum (*Sciaenops ocellatus*) to calcium and magnesium concentrations in fresh and salt water. Aquaculture 76:21-35.

Wurts, W. A. and R. R. Stickney. 1993. Growth rates of juvenile red drum *Sciaenops ocellatus* reared on commercial salmon feed in fresh and salt water. Journal of the World Aquaculture Society 24:422-424.

Zein-Eldin, Z. P., and D. V. Aldrich. 1963. Laboratory studies of shrimp tolerances to salinity and temperature, Proceedings of the Gulf and Caribbean Fisheries Institute, Coral Gables, Florida.

APPENDIX A

Generalized mathematical form of the triangle-wave algorithm. The algorithm functions identically for temperature and dissolved oxygen. Definitions of inputs, outputs, and relevant functions are provided for clarity. See text for narrative description of the triangle-wave algorithm.

Environment:

$E_{\min}$ = Minimum desired value of environment

$E_{\max}$ = Maximum desired value of environment

$E_{current}$ = Current value of environment

$E_{t_{increase}}$ = Target value of environment for increasing slope

$E_{t_{decrease}}$ = Target value of environment for decreasing slope

Time:

$T_{E_{\min}}$ = Time at which minimum desired value of environment will be achieved

$T_{E_{\max}}$ = Time at which maximum desired value of environment will be achieved

$T_{current}$ = Current time

Component functions:

$$\Delta T_{increase} = \left| T_{current} - T_{E_{\max}} \right| \qquad \Delta T_{decrease} = \left| T_{E_{\min}} - T_{current} \right|$$

$$Slope_{increase} = \frac{\left| E_{\max} - E_{\min} \right|}{\left| T_{E_{\max}} - T_{E_{\min}} \right|}$$

$$Slope_{decrease} = E_{\max} - (Slope_{increase} + Slope_{increase})$$

Algorithm:

IF $\left(T_{E_{\min}} > T_{current} < T_{E_{\max}} \right)$ THEN $E_{t_{increase}} = E_{\min} + (Slope_{increase} \times \Delta T_{increase})$

IF $\left(T_{E_{\min}} < T_{current} > T_{E_{\max}} \right)$ THEN $E_{t_{decrease}} = Slope_{decrease} - (Slope_{increase} \times \Delta T_{increase})$

APPENDIX B

Quick-reference list of reduced growth and modeling results across all experiments.

Chapter	Treatment			Observed Growth Rate (%/day)	MMSO	Winberg-adjustment
III	High DO	Cool (~19 °C)	LE	7.1	0.200	1.30
			HE	7.6	0.200	1.30
		Ambient (~25 °C)	LE	10.5	0.220	1.50
			HE	15.7	0.250	1.50
		Warm (~28 °C)	LE	12.2	0.260	1.50
			HE	18.9	0.250	1.50
IV	High DO	Low Temp	LE	2.93	0.110	2.00
			HE	3.98	0.185	1.10
		High Temp	LE	9.13	0.275	1.30
			HE	12.18	0.333	2.42
	Low Temp	Low DO	LE	2.23	0.196	1.32
			HE	4.34	0.200	1.15
		High DO	LE	2.53	0.085	2.00
			HE	5.85	0.201	1.05
	High Temp	Low DO	LE	5.18	0.266	1.62
			HE	13.58	0.242	1.54
		High DO	LE	6.43	0.315	1.72
			HE	20.39	0.338	2.40
V	Ambient O2		LE [a]	65.62	0.245	1.2
			HE	72.16	0.249	1.2
	O_2+ [b]		LE [a]	45.83	0.233	1.2
			HE	87.90	0.250	1.2

[a] Simulated with optimized model input for FeedEnergy in which LE diet had 15.9 kJ/g on a dry-weight basis.
[b] Simulated with optimized model input for ambient DO in which O_2+ = AmbO_2 + 2.0 mg/L.

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Fontaine, L. P., K. W. Whiteman, P. Li, G. S. Burr, K. A. Webb, J. Goff, D. M. Gatlin III, W. H. Neill, K. B. Davis, and R. R. Vega. 2007. Effects of temperature and feed energy on performance of juvenile red drum. Transactions of the American Fisheries Society 136:1193-1205.

Neill, W. H., T. S. Brandes, B. J. Burke, S. R. Craig, L. V. DiMichele, K A. Duchon, L. P. Fontaine, D. M. Gatlin III, C. Hutchings, R. E. Edwards, J. M. Miller, B. J. Ponwith, C. J. Stahl, J. R. Tomasso, and R. R. Vega. 2004. Ecophys.fish: a simulation model of fish growth in time-varying environmental regimes. Reviews in Fisheries Sciences 12:233-288.

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-Biologist, North Carolina Department of Transportation, Raleigh, NC, Present.
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-2003 Ecological Integration Symposium Organization Committee Member.
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Welsh Chemistry Scholar 1994
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